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(54) **INSECT-RESISTANT TRANSGENIC PLANTS**

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Related U.S. Patent Documents

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A01H 5/10 (2006.01)
C12N 15/82 (2006.01)
C12N 1/21 (2006.01)

(52) **U.S. Cl.** **800/302; 435/252.3; 435/418; 800/279**

(58) **Field of Classification Search** **800/302, 800/279, 292, 293, 294; 435/418, 252.3, 435/252.31, 469, 470; 536/23.71**
 See application file for complete search history.

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(57) **ABSTRACT**

The invention provides transgenic plants and transformed host cells which express modified cry 3B genes with enhanced toxicity to Coleopteran insects. Also disclosed are methods of making and using these transgenic plants, methods of making recombinant host cells expressing these δ -endotoxins, and methods of killing insects such as Colorado potato beetle (*Leptinotarsa decemlineata*), southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber) and western corn rootworm (*Diabrotica virgifera* LeConte).

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FIG. 1

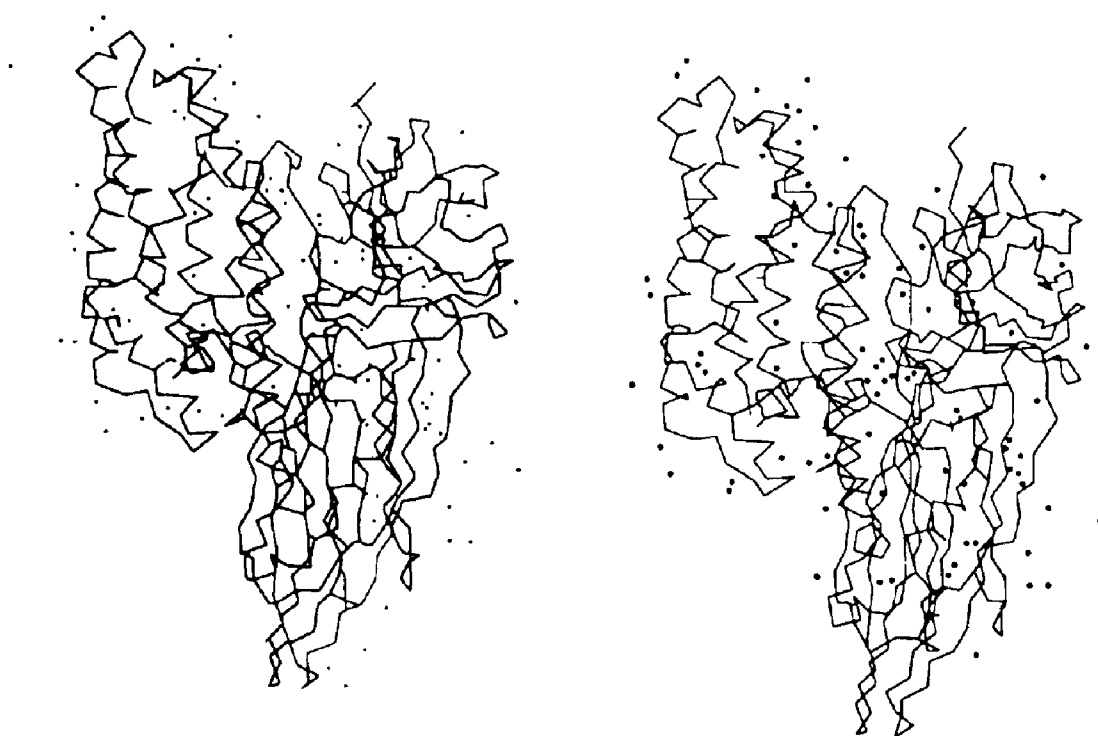


FIG. 2

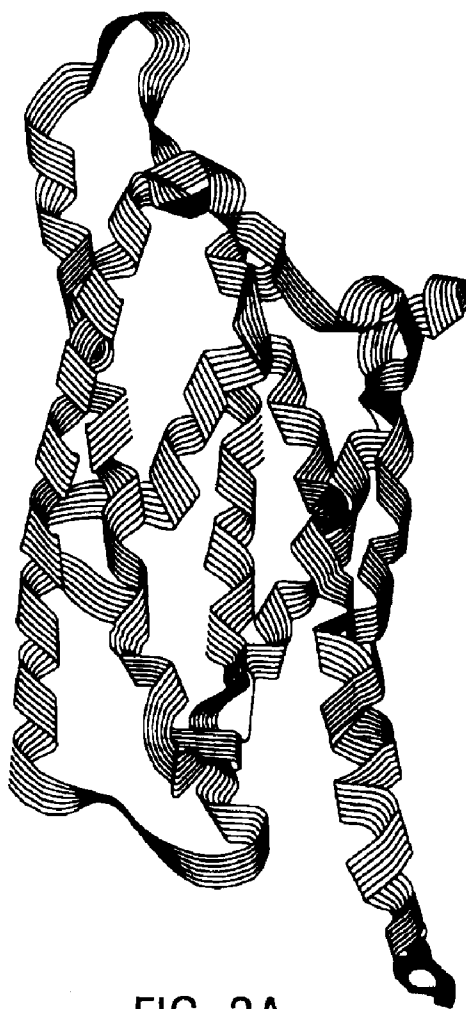


FIG. 3A

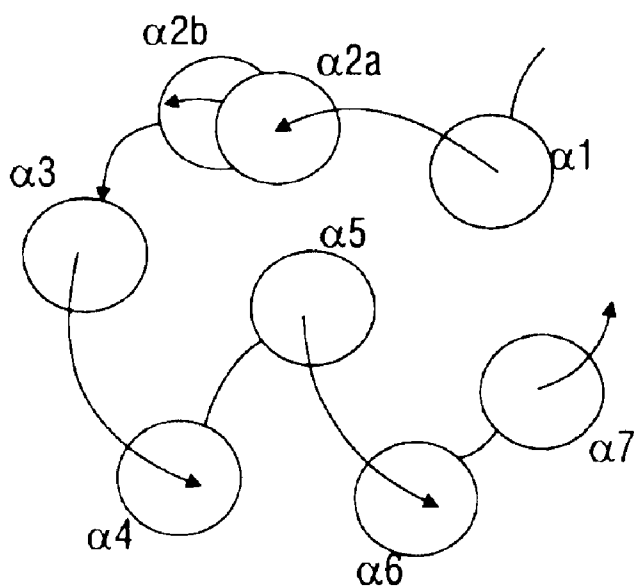


FIG. 3B

α helix	Amino acid Residues
$\alpha 1$	63-79
$\alpha 2a$	85-98
$\alpha 2b$	105-118
$\alpha 3$	124-153
$\alpha 4$	161-186
$\alpha 5$	194-215
$\alpha 6$	223-255
$\alpha 7$	260-286

FIG. 4

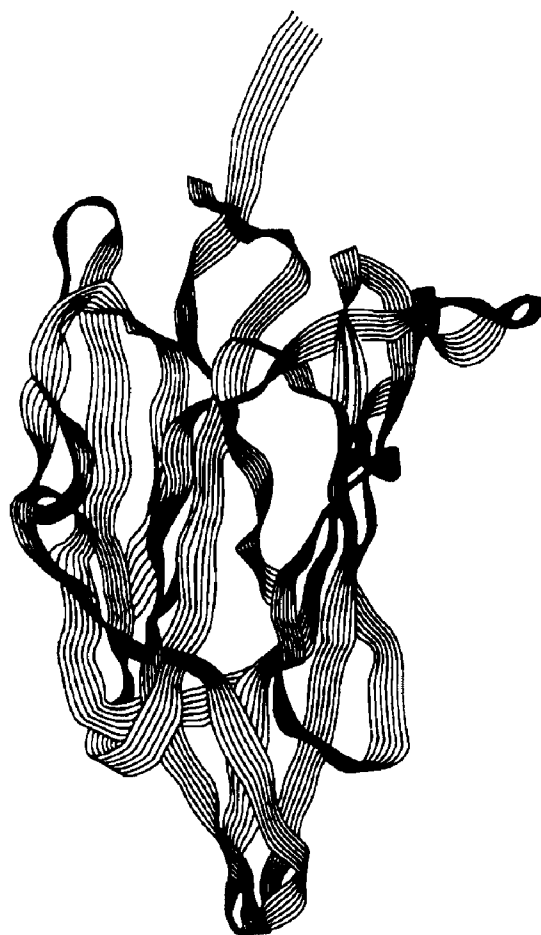


FIG. 5A

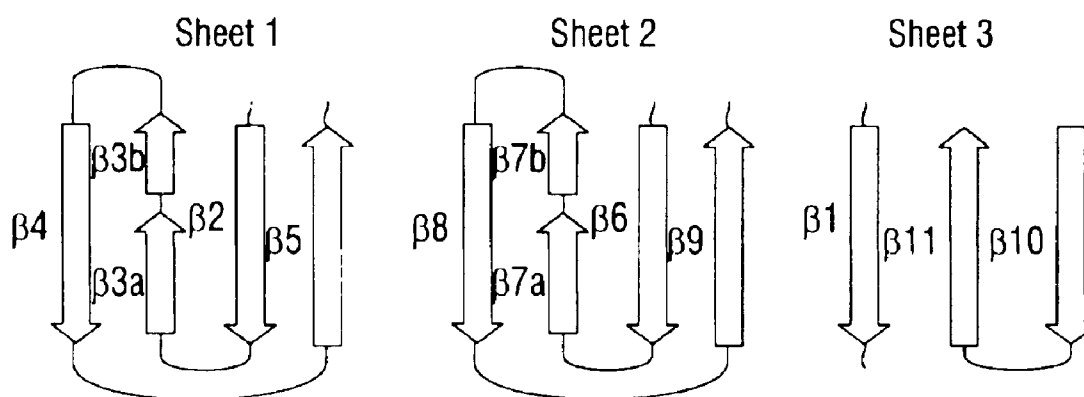


FIG. 5B

Sheet 1

β Strand	Amino Acid Residue
$\beta 2$	339-350
$\beta 3a$	256-360
$\beta 3b$	362-368
$\beta 4$	375-379
$\beta 5$	390-395

Sheet 2

β Strand	Amino Acid Residue
$\beta 6$	402-412
$\beta 7a$	416-419
$\beta 7b$	423-430
$\beta 8$	435-442
$\beta 9$	452-456

Sheet 3

β Strand	Amino Acid Residue
$\beta 1$	296-306
$\beta 10$	472-483
$\beta 11$	492-498

FIG. 6

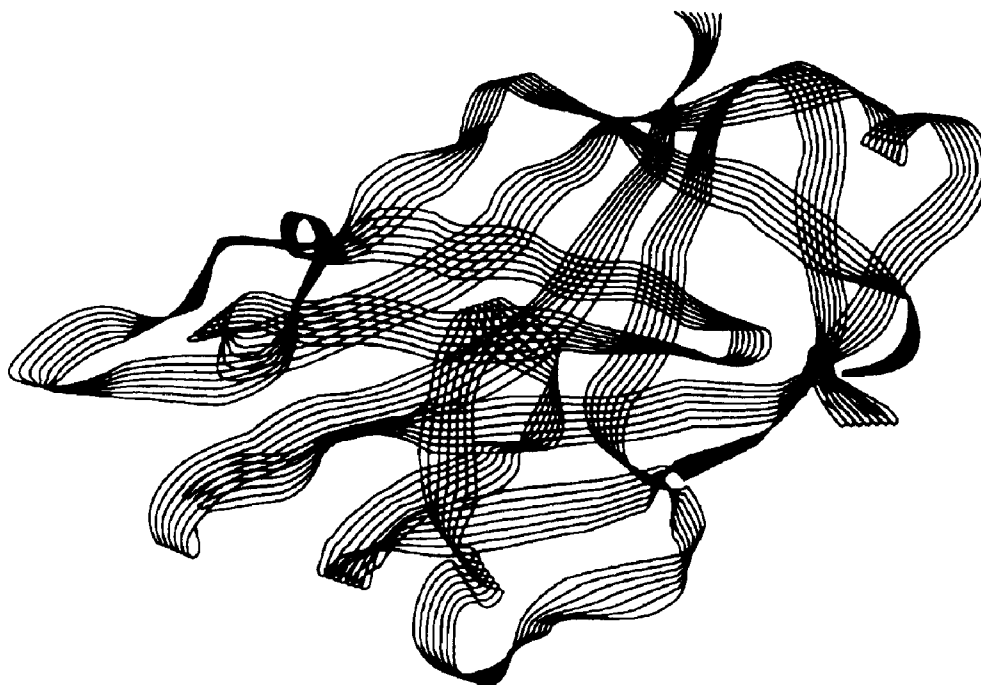


FIG. 7A

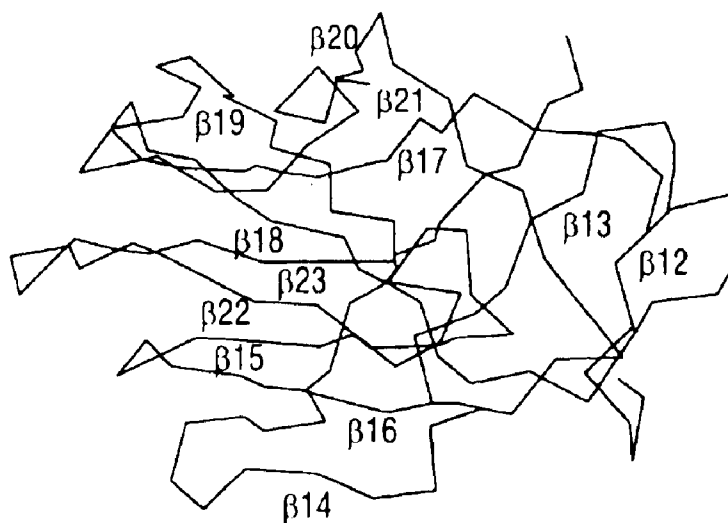


FIG. 7B

Strand Number	Amino Acid Residues
β 12	505-509
β 13	512-515
β 14	522-528
β 15	539-544
β 16	550-557
β 17	563-574
β 18	578-584
β 19	590-596
β 20	609-614
β 21	616-619
β 22	626-636
β 23	638-650

FIG. 8



FIG. 9A



FIG. 9B

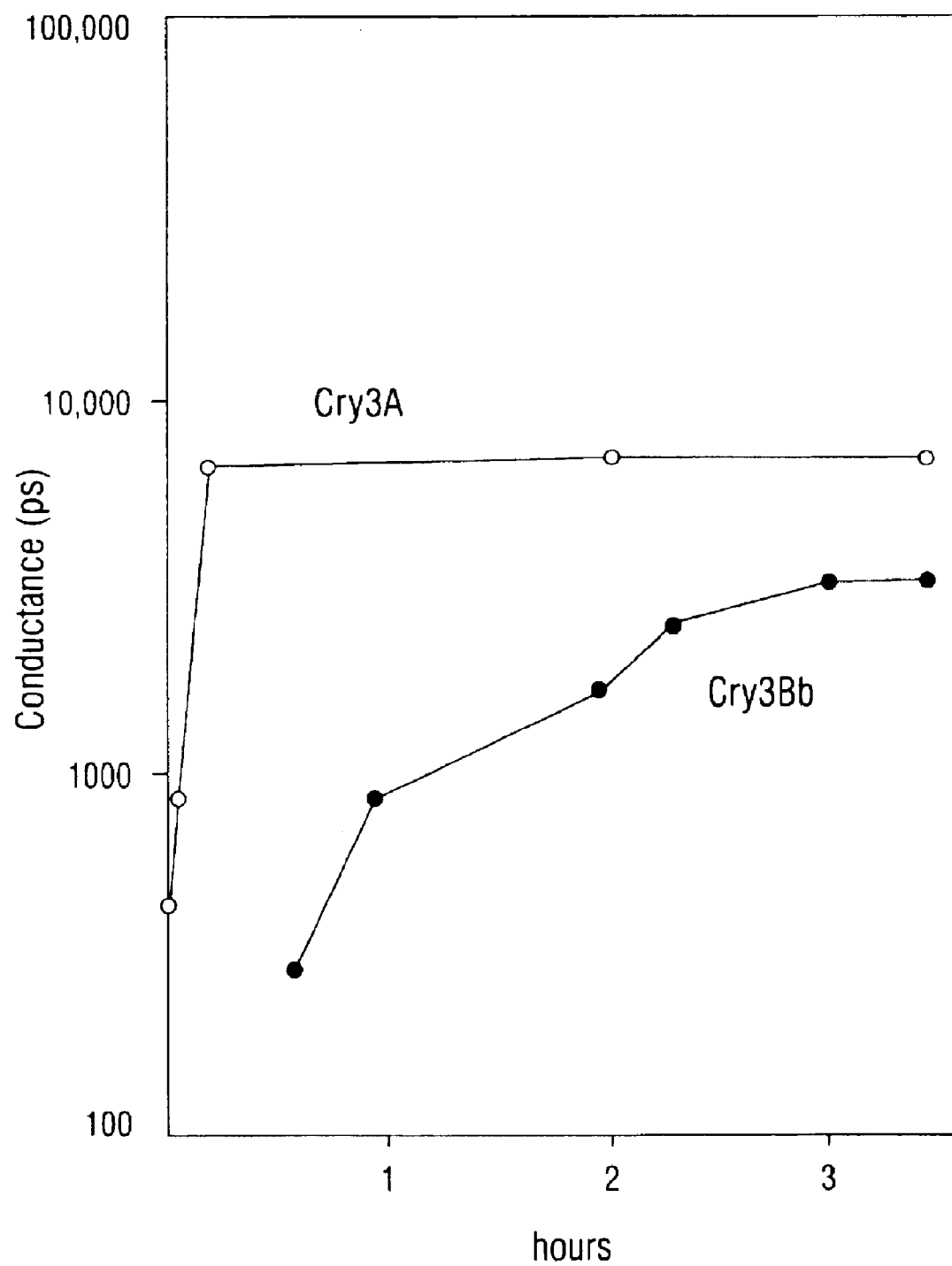


FIG. 10

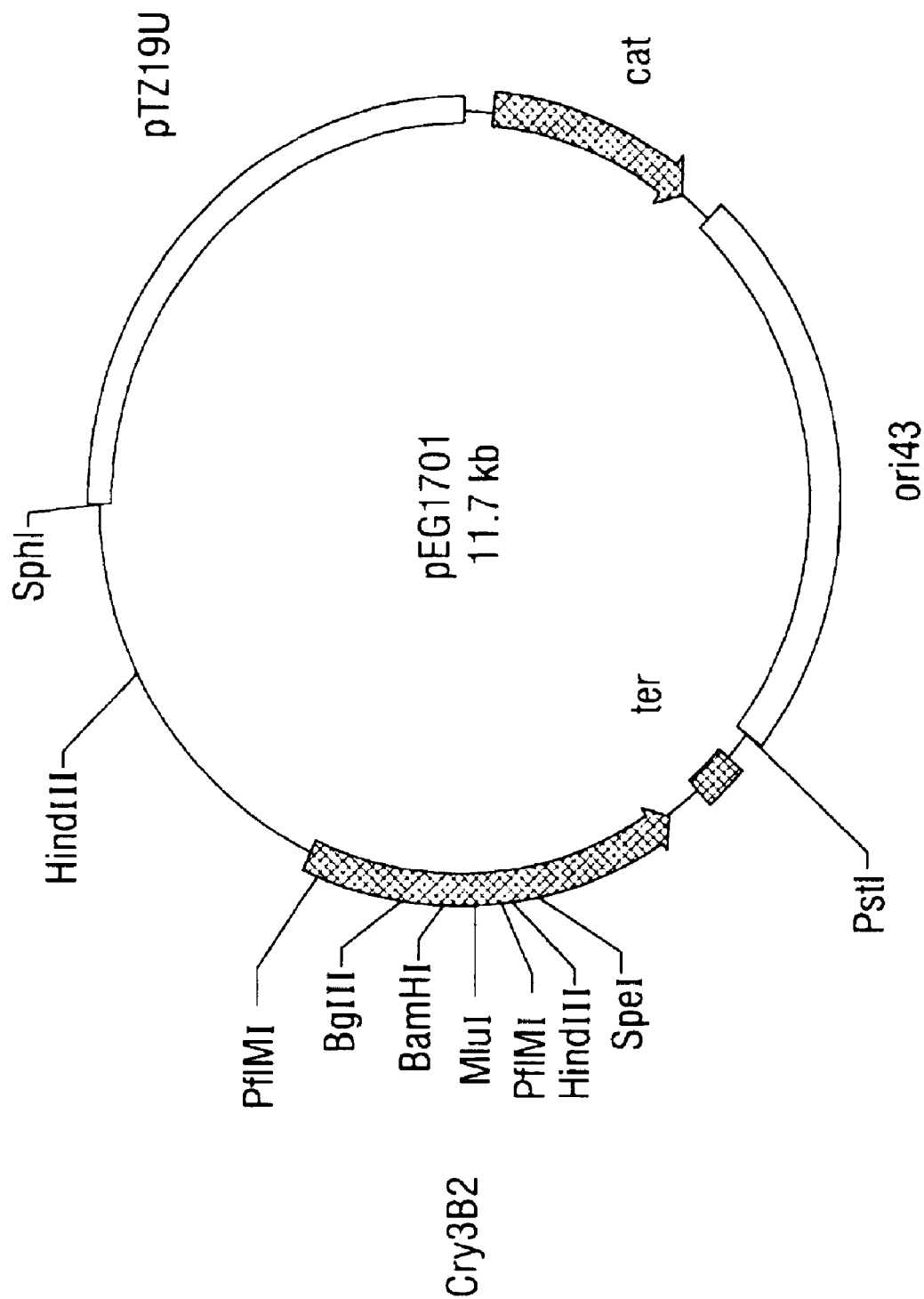


FIG. 11

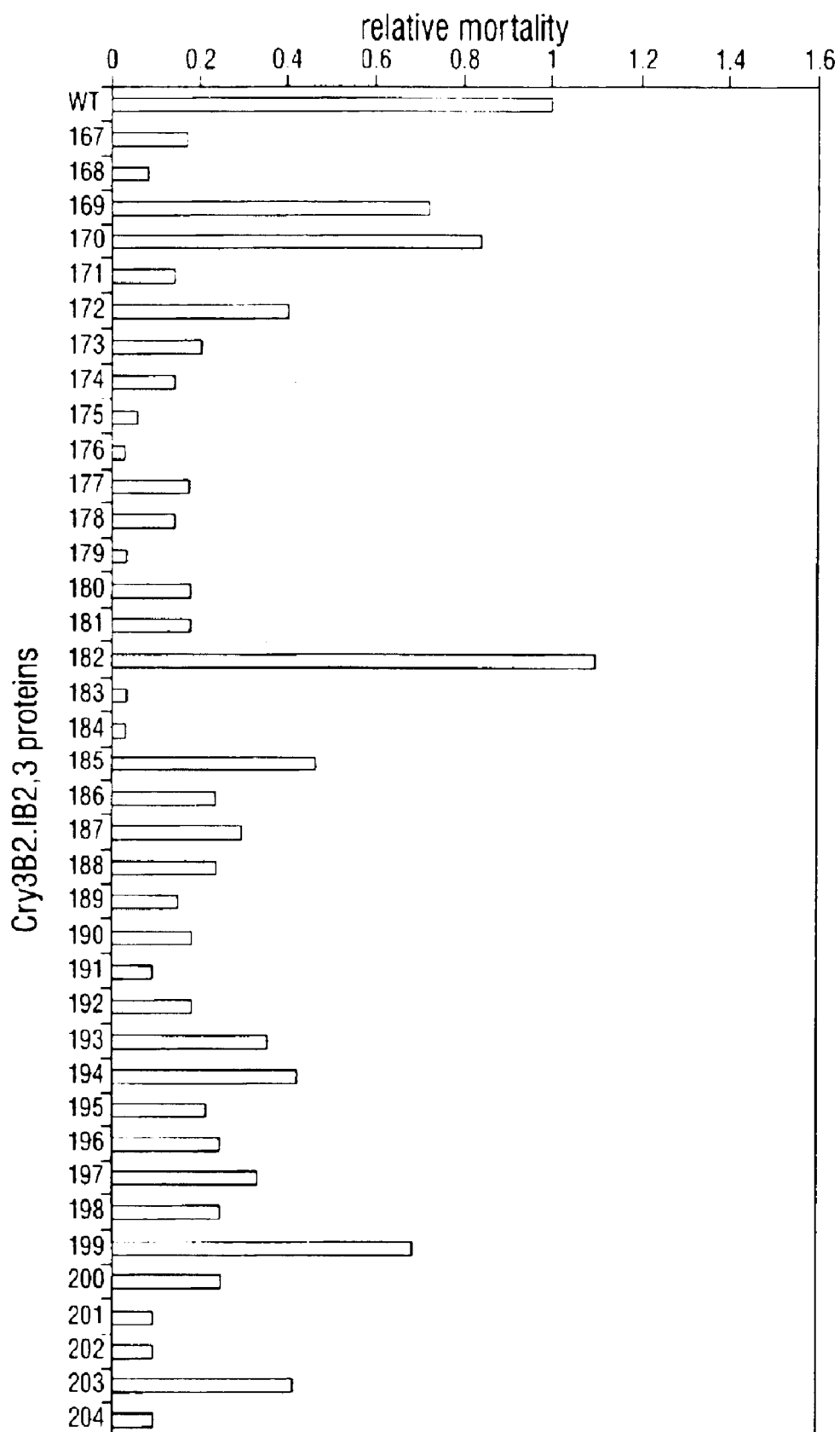


FIG. 12

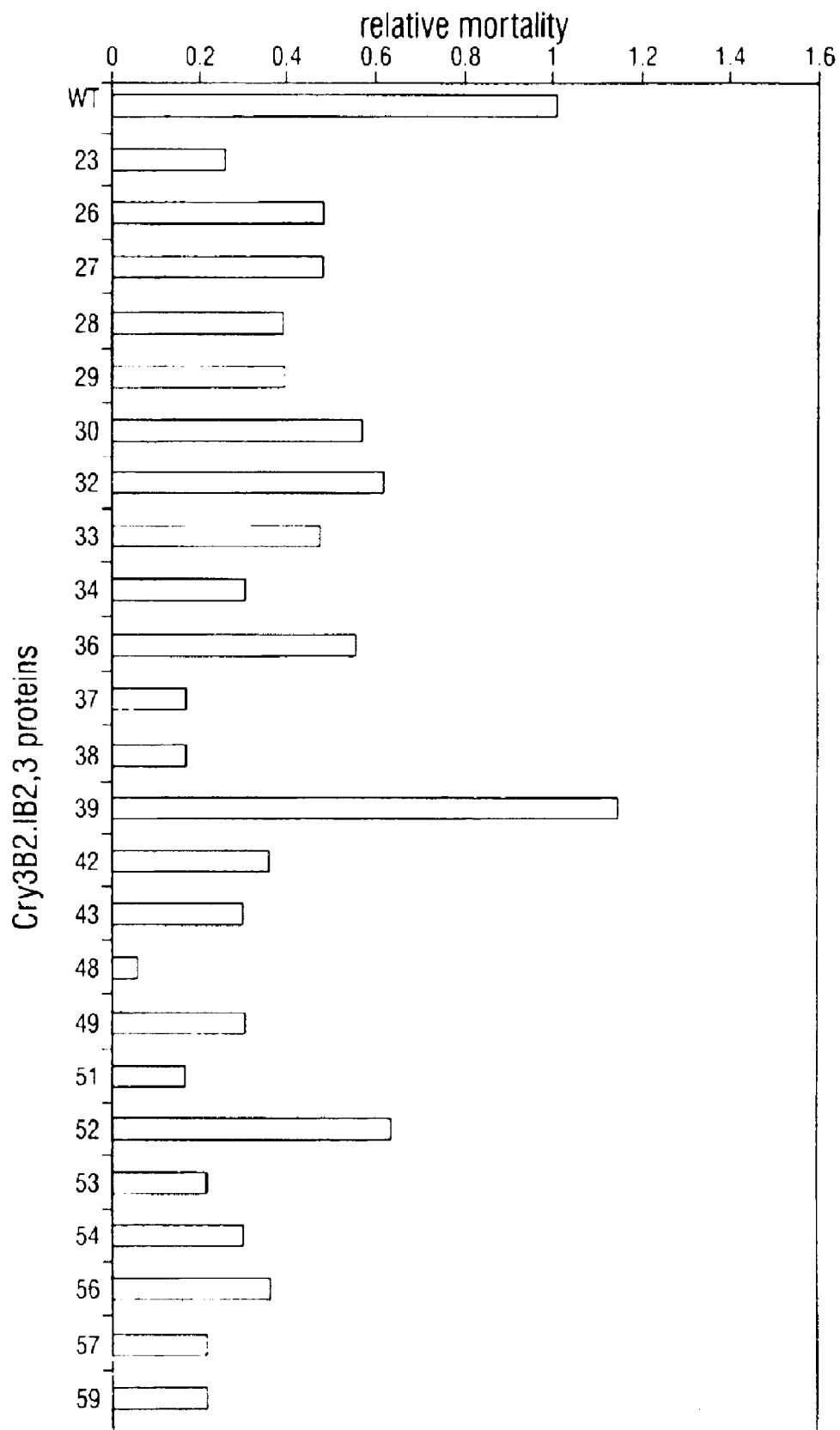


FIG. 13

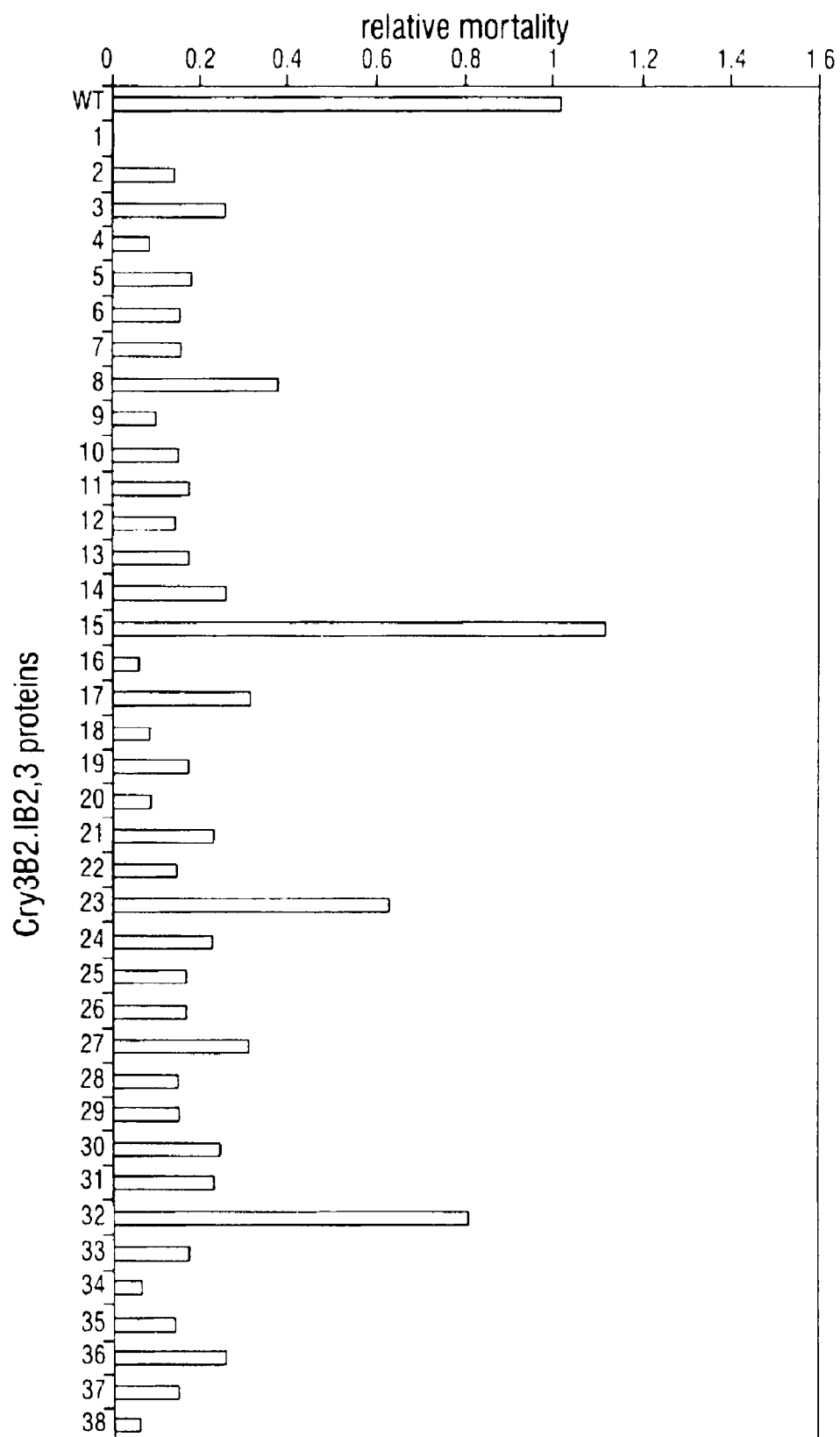


FIG. 14

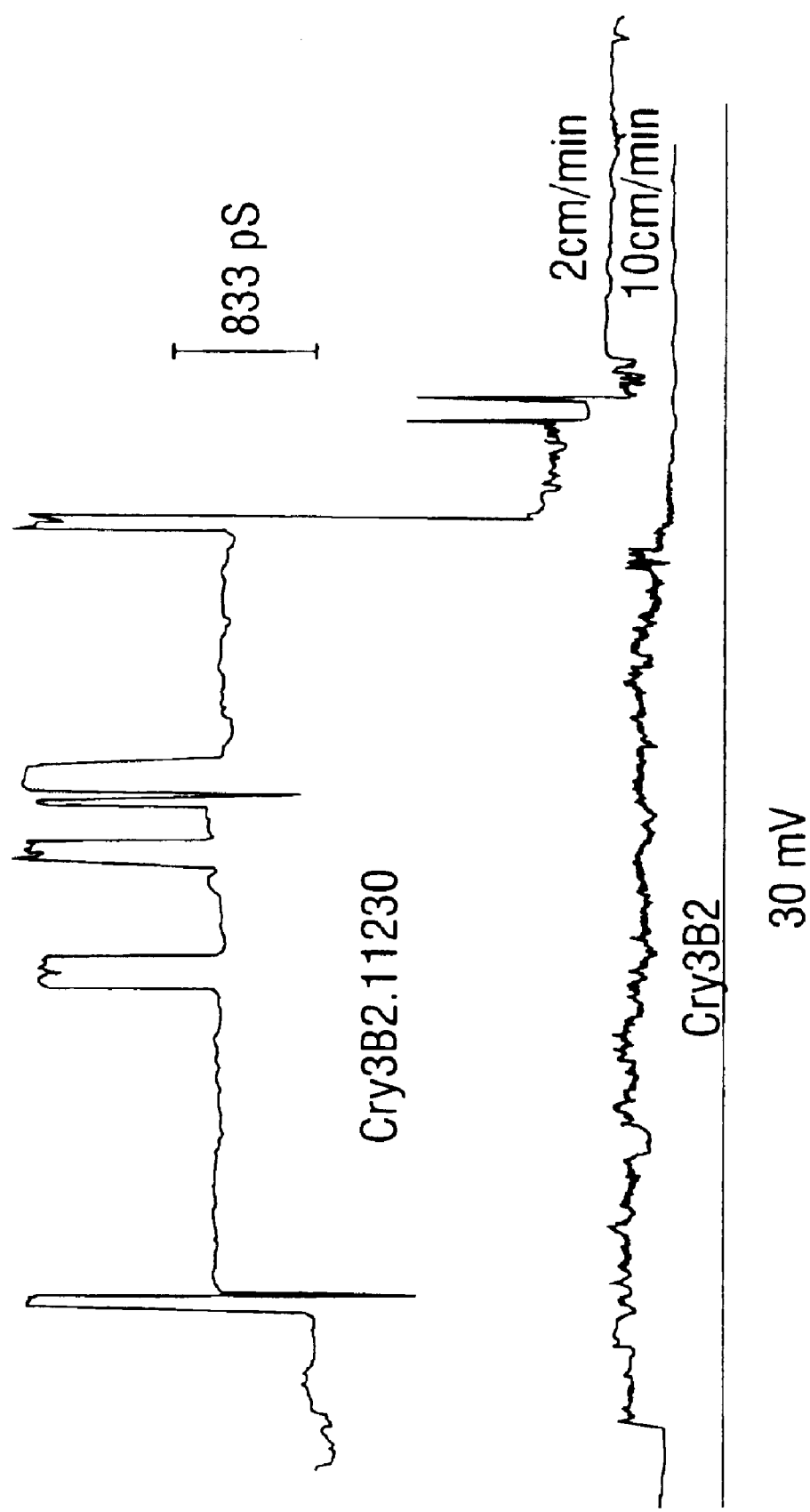


FIG. 15

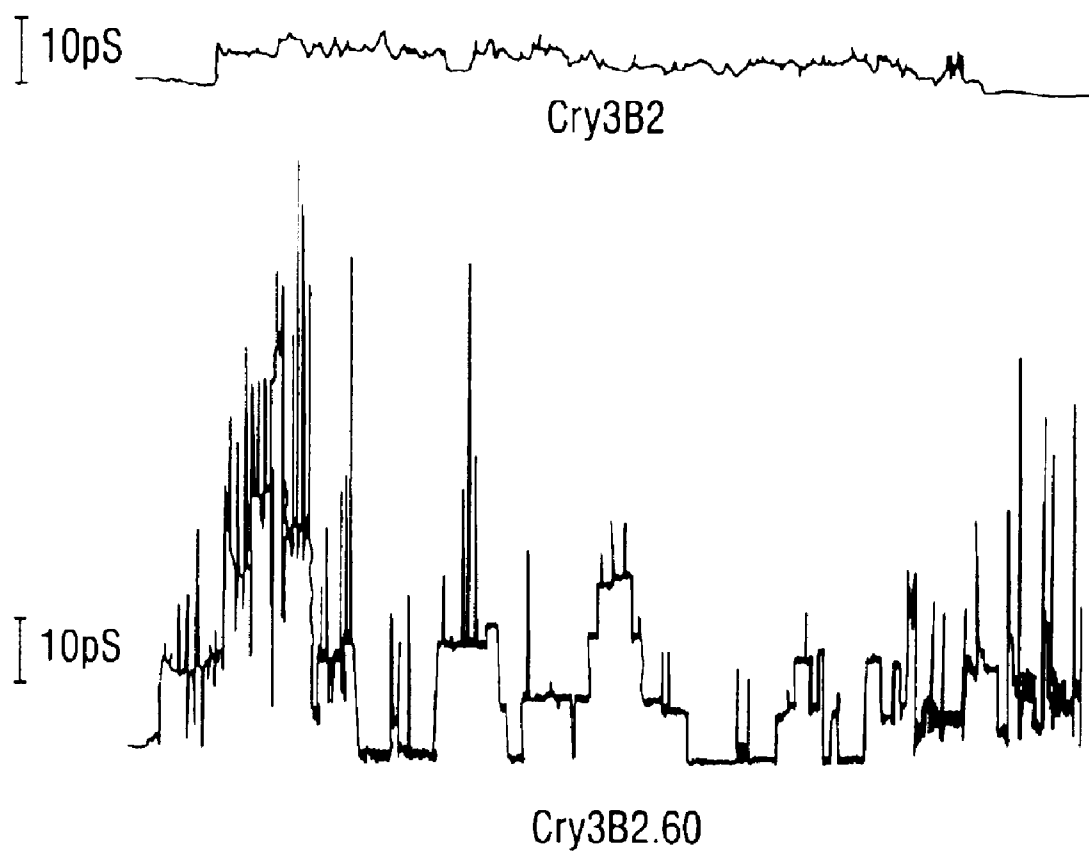


FIG. 16

ALIGNMENT OF CRY3 SEQUENCES

(Numbered according to Cry3BB)

(alpha helices underlined, beta sheets marked with + + +'s)

	1	10	20	30	40
CRY3C:	MNPNNRSEHDTI	ATENN	VNNHAQYPLADTP	- -	TLEELNY
CRY3BB:	MNPNNRSEHDTI	VTPNSELFTNHNQYPLADNP	NPSTLEELNY		
CRY3BB:	MNPNNRSEHDTI	VTPNSELFTNHNQYPLADNP	NPSTLEELNY		
CRY3BA:	MIRMGGRMNPNNRSEYDTI	VTPNSELFTNHNQYPLADNP	NPSTLEELNY		
CRY3A:	MIRKGGRMNPNNRSEHDTI	TTENN	VPTNHVQYPLAET	NPSTLEELNY	
	50	60	70	80	90
CRY3C:	KEFLRRTTDNNVEALDSSTT	DAIQIG	HTIGDILGVVLF	FPYGGALVSFY	
CRY3BB:	KEFLRMTEDDSTEVL	DNSTV	DAVGTGILVVGQILGVVGVP	FAGALTSFY	
CRY3BB:	KEFLRMTEDDSTEVL	DNSTV	DAVGTGILVVGQILGVVGVP	FAGALTSFY	
CRY3BA:	KEFLRMTADNSTEVLDSSTV	DAVGTGILVVGQILGVVGVP	FAGALTSFY		
CRY3A:	KEFLRMTADNTEALDSSTT	<u>DVIGIGILVVGQILGVVLF</u>	<u>FPYGGALVSFY</u>		
	100	110	120	130	140
CRY3C:	INFLNTIWPSE	-DPWKA	MQVEALMDQI	IADYAK	NFATAELQGLQNNFE
CRY3BB:	QSFLNTIWPSEADPWKA	MAQVEVLIDH	IEEYAKSK	ALAE	LQGLQNNFE
CRY3BB:	QSFLNTIWPSEADPWKA	MAQVEVLIDH	IEEYAKSK	ALAE	LQGLQNNFE
CRY3BA:	QSFLNTIWPSEADPWKA	MAQVEVLIDH	IEEYAKSK	ALAE	LQGLQNNFE
CRY3A:	<u>INFLNTIWPSE</u>	<u>-DPWKA</u>	<u>MEQVEALMDQI</u>	<u>IADYAK</u>	<u>NFATAELQGLQNNFE</u>
	150	160	170	180	190
CRY3C:	DYVSALDSWKE	TPLTL	RDGR	EQGRIRELFSQAESH	FRNMPSEFAVSGYEV
CRY3BB:	DYVNALNSWKE	TPLSL	RSKRQDR	IRELFSQAESH	FRNMPSEFAVSKFEV
CRY3BB:	DYVNALNSWKE	TPLSL	RSKRQDR	IRELFSQAESH	FRNMPSEFAVSKFEV
CRY3BA:	DYVNALDSWKE	APVNL	RSRRSQDR	IRELFSQAESH	FRNMPSEFAVSKFEV
CRY3A:	<u>DYVSALSSWKE</u>	<u>NPVSSRNPH</u>	<u>EQGRIRELFSQAESH</u>	<u>FRNMPSEFAVSGYEV</u>	

FIG. 17A

AMENDED

	200	210	220	230	240
CRY3C:	LFLPTYAQAANTH	LLLLKDAQIYGT	DWGYSTDDLNEF	HTKQKDLTIE	YTN
CRY3BB2:	LFLPTYAQAANTH	LLLLKDAQVFGE	EWGYSSDVAEF	YHRQLKLTQQ	YTD
CRY3BB:	LFLPTYAQAANTH	LLLLKDAQVFGE	EWGYSSDVAEF	YHRQLKLTQQ	YTD
CRY3BA:	LFLPTYAQAANTH	LLLLKDAQVFGE	EWGYSSDIAEF	YQRQLKLTQQ	YTD
CRY3A:	<u>LFLTTYAQAANTH</u>	<u>LFLKDAQIYGE</u>	<u>EWGYEKEDIAEF</u>	<u>YKRQLKLTQE</u>	<u>YTD</u>

	250	260	270	280	290
CRY3C:	HCAKWKAGLDK	LRGSTYEEWVK	FNRYRREMTLT	VLDLITLFP	LYDVRTY
CRY3BB2:	HCVNWYNVGLN	LRGSTYDAWVK	FNRFREMTLT	VLDLIVLFP	PFYDVRLY
CRY3BB:	HCVNWYNVGLN	LRGSTYDAWVK	FNRFREMTLT	VLDLIVLFP	PFYDIRLY
CRY3BA:	HCVNWYNVGLN	SLRGSTYDAWVK	FNRFREMTLT	VLDLIVLFP	PFYDVRLY
CRY3A:	<u>HCVKWYNVGLD</u>	<u>KLRGSSYESW</u>	<u>VNFNRYRREMT</u>	<u>LTVLDLIALFP</u>	<u>LYDVRLY</u>

	300	310	320	330	340
CRY3C:	TKGVKTELTRD	VLTDPIVAVNN	MNGYGTTFSNI	ENYIRKPHLFD	YLHAIQ
CRY3BB2:	SKGVKTELTRD	IFTDPIFSLNT	LQEYGPTFLSI	ENSIRKPHLFD	YLQGIE
CRY3BB:	SKGVKTELTRD	IFTDPIFSLNT	LQEYGPTFLSI	ENSIRKPHLFD	YLQGIE
CRY3BA:	SKGVKTELTRD	IFTDPIFTLNAL	QEYGPTFSSI	ENSIRKPHLFD	YLRGIE
CRY3A:	PKEVKTELTRD	VLTDPIGVNNL	RGYGTTFSNI	ENYIRKPHLFD	YLHRIQ
	+++++				++++

	350	360	370	380	390
CRY3C:	FHSRLQPGYFG	TDSFNYWSGNY	VSTRSSIGSDEI	IRSPFYGNKST	LDVQN
CRY3BB2:	FHTRLQPGYSG	KDSFNYWSGNY	VETRPSIGSSKIT	ITSPFYGDKSTE	PVQK
CRY3BB:	FHTRLQPGYFG	KDSFNYWSGNY	VETRPSIGSSKIT	ITSPFYGDKSTE	PVQK
CRY3BA:	FHTRLQPGYSG	KDSFNYWSGNY	VETRPSIGSNDT	ITSPFYGDKSIE	PIQK
CRY3A:	FHTRFQPGYYG	NDSFNYWSGNY	VSTRPSIGSNDI	ITSPFYGNKSSEP	VQN
	+++++	++++	+++++	++++	+++

	400	410	420	430	
CRY3C:	LEFNGEKVFR	AVANGNLAVWP	VGTTGGTKIH	SGVTKVQFSQ	YNDRKDEVRT
CRY3BB2:	LSFDGQKVYR	TIANTDVAAPNG	----	KIYFGVTKVDF	SQYDDQKNETST
CRY3BB:	LSFDGQKVYR	TIANTDVAAPNG	----	KVYLGVTKVDF	SQYDDQKNETST
CRY3BA:	LSFDGQKVYR	TIANTDIAAFPDG	----	KIYFGVTKVDF	SQYDDQKNETST
CRY3A:	LEFNGEKVYRA	VANTNLAVWPSA	----	VYSGVTKVEFSQ	YNDQTDEAST
	+++	+++++	++++	+++++	++++

FIG. 17B

	440	450	460	470	480
CRY3C:	QTYDSKRLVGGIV-FDSIQQLPPTTDEPLEAYSHQLNVAQFLQSSR				
CRY3BB2:	QTYDSKRLVGGIVVAQDSIQQLPPTTDEPLEAYSHQLNVAQFLMQRR				
CRY3BB:	QTYDSKRLVGGIVVAQDSIQQLPPTTDEPLEAYSHQLNVAQFLMQRR				
CRY3BA:	QTYDSKRLVGGIVVAQDSIQQLPPTTDEPLEAYSHQLNVAQFLMQRR				
CRY3A:	QTYDSKRLVGGIV-FDSIQQLPPTTDEPLEAYSHQLNVAQFLMQRR				
	++++	+++++			++++++
	490	500	510	520	530
CRY3C:	GTIPVETWTHRSVDFFNTIDAEKITQLPVVAYALSSGASIEGPGFTGG				
CRY3BB2:	GTIPVETWTHRSVDFFNTIDAEKITQLPVVAYALSSGASIEGPGFTGG				
CRY3BB:	GTIPVETWTHRSVDFFNTIDAEKITQLPVVAYALSSGASIEGPGFTGG				
CRY3BA:	GTIPVETWTHRSVDFFNTIDAEKITQLPVVAYALSSGASIEGPGFTGG				
CRY3A:	GTIPVETWTHRSVDFFNMIDSKKITQLPVVAYALSSGASIEGPGFTGG				
	+-----	++++	++++		++++++
	540	550	560	570	580
CRY3C:	DII-EKCT-NGSGLTLYVTPAPDLTYSKTYKIRIRYASTSQVREGIDLSY				
CRY3BB2:	NLEFLFESSNSIAEFKVTL-NSAALLQRYRVRIYASTTNLEFVQNSNN				
CRY3BB:	NLEFLFESSNSIAEFKVTL-NSAALLQRYRVRIYASTTNLEFVQNSNN				
CRY3BA:	NLEFLFESSNSIAEFKVTL-NSAALLQRYRVRIYASTTNLEFVQNSNN				
CRY3A:	DII-EKCT-NGSGLTLYVTPAPDLTYSKTYKIRIRYASTSQVREGIDLSY				
	++++++	++++++	++++++	++++++	++++++
	590	600	610	620	630
CRY3C:	THSIGYFDITMDKUNTLTYNSFNLSVSAPITSG-GNIGVSVGGIGSG				
CRY3BB2:	DFIVLYINKTMNIDGDLTYQTFGLATTNSNMGFSGDNI LIIGAESFVSN				
CRY3BB:	DFIVLYINKTMNIDGDLTYQTFGLATTNSNMGFSGDNI LIIGAESFVSN				
CRY3BA:	DFIVLYINKTMNIDGDLTYQTFGLATTNSNMGFSGDNI LIIGAESFVSN				
CRY3A:	DFIVLYINKTMNIDGDLTYQTFGLATTNSNMGFSGDNI LIIGAESFVSN				
	++++++	++++++	++++		++++++
	640	650			
CRY3C:	DEVYIDIEFFIPMD				
CRY3BB2:	ENIYIDIEFFIPVQL				
CRY3BB:	ENIYIDIEFFIPVQL				
CRY3BA:	ENIYIDIEFFIPVQ				
CRY3A:	DEVYIDIEFFIPVN				
	+++++				

FIG. 17C

INSECT-RESISTANT TRANSGENIC PLANTS

Matter enclosed in heavy brackets [] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.

1.0 BACKGROUND OF THE INVENTION

1.1 Field of the Invention

This invention relates to transgenic plants which express bacterially-derived proteins which are toxic to Coleopteran insects such as Colorado potato beetle (*Leptinotarsa decemlineata*), southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber) and western corn rootworm (*Diabrotica virgifera virgifera* LeConte).

1.2 Description of the Related Art

Almost all field crops, plants, and commercial farming areas are susceptible to attack by one or more insect pests. Particularly problematic are Coleopteran and Lepidoptern pests. For example, vegetable and cole crops such as artichokes, kohlrabi, arugula, leeks, asparagus, lentils, beans, lettuce (e.g., head, leaf, romaine), beets, bok choy, malanga, broccoli, melons (e.g., muskmelon, watermelon, crenshaw, honeydew, cantaloupe), brussels sprouts, cabbage, cardoni, carrots, napa, cauliflower, okra, onions, celery, parsley, chick peas, parsnips, chicory, peas, chinese cabbage, peppers, collards, potatoes, cucumber, pumpkins, cucurbits, radishes, dry bulb onions, rutabaga, eggplant, salsify, escarole, shallots, endive, soybean, garlic, spinach, green onions, squash, greens, sugar beets, sweet potatoes, turnip, swiss chard, horseradish, tomatoes, kale, turnips, and a variety of spices are sensitive to infestation by one or more of the following insect pests: alfalfa looper, armyworm, beet armyworm, artichoke plume moth, cabbage budworm, cabbage looper, cabbage webworm, corn earworm, celery leafeater, cross-striped cabbageworm, european corn borer, diamondback moth, green cloverworm, imported cabbageworm, melonworm, omnivorous leafroller, pickleworm, rindworm complex, saltmarsh caterpillar, soybean looper, tobacco budworm, tomato fruitworm, tomato horuworm, tomato pinworm, velvetbean caterpillar, and yellowstriped armyworm. Likewise, pasture and hay crops such as alfalfa, pasture grasses and silage are often attacked by such pests as armyworm, beef armyworm, alfalfa caterpillar, European skipper, a variety of loopers and webworms, as well as yellowstriped armyworms.

Fruit and vine crops such as apples, apricots, cherries, nectarines, peaches, pears, plums, prunes, quince almonds, chestnuts, filberts, pecans, pistachios, walnuts, citrus, blackberries, blueberries, boysenberries, cranberries, currants, loganberries, raspberries, strawberries, grapes, avocados, bananas, kiwi, persimmons, pomegranate, pineapple, tropical fruits are often susceptible to attack and defoliation by achema sphinx moth, amorbia, armyworm, citrus cutworm, banana skipper, blackheaded fireworm, blueberry leafroller, cankerworm, cherry fruitworm, citrus cutworm, cranberry girdler, eastern tent caterpillar, fall webworm, fall webworm, filbert leafroller, filbert webworm, fruit tree leafroller, grape berry moth, grape leafroller, grapeleaf skeletonizer, green fruitworm, gummosisbetrachtedra commosae, gypsy moth, hickory shuckworm, hornworms, loopers, navel orangeworm, obliquebanded leafroller, omnivorous leafroller, omnivorous looper, orange tortrix, orangedog, oriental fruit moth, pandemis leafroller,

peach twig borer, pecan nut casebearer, redbanded leafroller, redhumped caterpillar, roughskinned cutworm, saltmarsh caterpillar, spanworm, tent caterpillar, thecla-thecla basillides, tobacco budworm, tortrix moth, tufted apple budmoth, variegated leafroller, walnut caterpillar, western tent caterpillar, and yellowstriped armyworm.

Field crops such as canola/rape seed, evening primrose, meadow foam, corn (field, sweet, popcorn), cotton, hops, jojoba, peanuts, rice, safflower, small grains (barley, oats, rye, wheat, etc.), sorghum, soybeans, sunflowers, and tobacco are often targets for infestation by insects including armyworm, asian and other corn borers, banded sunflower moth, beet armyworm, bollworm, cabbage looper, corn rootworm (including southern and western varieties), cotton leaf perforator, diamondback moth, european corn borer, green cloverworm, headmoth, headworm, imported cabbageworm, loopers (including *Anacamptodes* spp.), obliquebanded leafroller, omnivorous leaftier, podworm, podworm, saltmarsh caterpillar, southwestern corn borer, soybean looper, spotted cutworm, sunflower moth, tobacco budworm, tobacco hornworm, velvetbean caterpillar,

Bedding plants, flowers, ornamentals, vegetables and container stock are frequently fed upon by a host of insect pests such as armyworm, azalea moth, beet armyworm, diamondback moth, ello moth (hornworm), Florida fern caterpillar, Io moth, loopers, oleander moth, omnivorous leafroller, omnivorous looper, and tobacco budworm.

Forests, fruit, ornamental, and nut-bearing trees, as well as shrubs and other nursery stock are often susceptible to attack from diverse insects such as bagworm, blackheaded budworm, browntail moth, california oakworm, douglas fir tussock moth, elm spanworm, fall webworm, fruittree leafroller, greenstriped mapleworm, gypsy moth, jack pine budworm, mimosa webworm, pine butterfly, redhumped caterpillar, saddleback caterpillar, saddle prominent caterpillar, spring and fall cankerworm, spruce budworm, tent caterpillar, tortrix, and western tussock moth. Likewise, turf grasses are often attacked by pests such as armyworm, sod webworm, and tropical sod webworm.

Because crops of commercial interest are often the target of insect attack, environmentally-sensitive methods for controlling or eradicating insect infestation are desirable in many instances. This is particularly true for farmers, nurserymen, growers, and commercial and residential areas which seek to control insect populations using eco-friendly compositions.

The most widely used environmentally-sensitive insecticidal formulations developed in recent years have been composed of microbial pesticides derived from the bacterium *Bacillus thuringiensis*. *B. thuringiensis* is a Gram-positive bacterium that produces crystal proteins or inclusion bodies which are specifically toxic to certain orders and species of insects. Many different strains of *B. thuringiensis* have been shown to produce insecticidal crystal proteins. Compositions including *B. thuringiensis* strains which produce insecticidal proteins have been commercially-available and used as environmentally-acceptable insecticides because they are quite toxic to the specific target insect, but are harmless to plants and other non-targeted organisms.

1.2.1 δ -Endotoxins

δ -endotoxins are used to control a wide range of leaf-eating caterpillars and beetles, as well as mosquitoes. These proteinaceous parasporal crystals, also referred to as insecticidal crystal proteins, crystal proteins, Bt inclusions, crystalline inclusions, inclusion bodies, and Bt toxins, are a large

collection of insecticidal proteins produced by *B. thuringiensis* that are toxic upon ingestion by a susceptible insect host. Over the past decade research on the structure and function of *B. thuringiensis* toxins has covered all of the major toxin categories, and while these toxins differ in specific structure and function, general similarities in the structure and function are assumed. Based on the accumulated knowledge of *B. thuringiensis* toxins, a generalized mode of action for *B. thuringiensis* toxins has been created and includes: ingestion by the insect, solubilization in the insect midgut (a combination stomach and small intestine), resistance to digestive enzymes sometimes with partial digestion actually "activating" the toxin, binding to the midgut cells, formation of a pore in the insect cells and the disruption of cellular homeostasis (English and Slatin, 1992).

1.2.2 Genes Encoding Crystal Proteins

Many of the δ -endotoxins are related to various degrees by similarities in their amino acid sequences. Historically, the proteins and the genes which encode them were classified based largely upon their spectrum of insecticidal activity. The review by Höfte and Whiteley (1989) discusses the genes and proteins that were identified in *B. thuringiensis* prior to 1990, and sets forth the nomenclature and classification scheme which has traditionally been applied to *B. thuringiensis* genes and proteins. *cryI* genes encode lepidopteran-toxic *CryI* proteins. *cryII* genes encode *CryII* proteins that are toxic to both lepidopterans and dipterans. *cryIII* genes encode coleopteran-toxic *CryIII* proteins, while *cryIV* genes encode dipteran-toxic *CryIV* proteins.

Based on the degree of sequence similarity, the proteins were further classified into subfamilies; more highly related proteins within each family were assigned divisional letters such as *CryIA*, *CryIB*, *CryIC*, etc. Even more closely related proteins within each division were given names such as *CryIC1*, *CryIC2*, etc. Recently a new nomenclature was developed which systematically classified the *Cry* proteins based upon amino acid sequence homology rather than upon insect target specificities. The classification scheme for many known toxins, not including allelic variations in individual proteins, is summarized in Table 1.

TABLE 1

KNOWN <i>B. THURINGIENSIS</i> δ -ENDOTOXINS, GENBANK ACCESSION NUMBERS, AND REVISED, NOMENCLATURE ^A		
New	Old	Genbank Accession #
<i>CryIAa1</i>	<i>CryIA(s)</i>	M11250
<i>CryIAa2</i>	<i>CryIA(s)</i>	M10917
<i>CryIAa3</i>	<i>CryIA(s)</i>	D00348
<i>CryIAa4</i>	<i>CryIA(s)</i>	X13535
<i>CryIAa5</i>	<i>CryIA(a)</i>	D17518
<i>CryIAa6</i>	<i>CryIA(a)</i>	U43605
<i>CryIAb1</i>	<i>CryIA(b)</i>	M13898
<i>CryIAb2</i>	<i>CryIA(b)</i>	M12661
<i>CryIAb3</i>	<i>CryIA(b)</i>	M15271
<i>CryIAb4</i>	<i>CryIA(b)</i>	D00117
<i>CryIAb5</i>	<i>CryIA(b)</i>	X04698
<i>CryIAb6</i>	<i>CryIA(b)</i>	M37263
<i>CryIAb7</i>	<i>CryIA(b)</i>	X13233
<i>CryIAb8</i>	<i>CryIA(b)</i>	M16463
<i>CryIAb9</i>	<i>CryIA(b)</i>	X54939
<i>CryIAb10</i>	<i>CryIA(b)</i>	
<i>CryIAc1</i>	<i>CryIA(c)</i>	M11068
<i>CryIAc2</i>	<i>CryIA(c)</i>	M35524
<i>CryIAc3</i>	<i>CryIA(c)</i>	X54159
<i>CryIAc4</i>	<i>CryIA(c)</i>	M73249

TABLE 1-continued

KNOWN <i>B. THURINGIENSIS</i> δ -ENDOTOXINS, GENBANK ACCESSION NUMBERS, AND REVISED, NOMENCLATURE ^A		
New	Old	Genbank Accession #
<i>CryIAc5</i>	<i>CryIA(c)</i>	M73248
<i>CryIAc6</i>	<i>CryIA(c)</i>	U43606
<i>CryIAc7</i>	<i>CryIA(c)</i>	U87793
<i>CryIAc8</i>	<i>CryIA(c)</i>	U87397
<i>CryIAc9</i>	<i>CryIA(c)</i>	U89872
<i>CryIAc10</i>	<i>CryIA(c)</i>	AJ002514
<i>CryIAd1</i>	<i>CryIA(d)</i>	M73250
<i>CryIAe1</i>	<i>CryIA(c)</i>	M65252
<i>CryIBa1</i>	<i>CryIB</i>	X06711
<i>CryIBa2</i>		X95704
<i>CryIBb1</i>	ET5	L32020
<i>CryIBc1</i>	<i>CryIh(c)</i>	Z46442
<i>CryIBd1</i>	<i>CryEI</i>	
<i>CryICa1</i>	<i>CryIC</i>	X07518
<i>CryICa2</i>	<i>CryIC</i>	X13620
<i>CryICa3</i>	<i>CryIC</i>	M73251
<i>CryICa4</i>	<i>CryIC</i>	A27642
<i>CryICa5</i>	<i>CryIC</i>	X96682
<i>CryICo6</i>	<i>CryIC</i>	X96683
<i>CryICa7</i>	<i>CryIC</i>	X96684
<i>CryICb1</i>	<i>CryIC(b)</i>	M97880
<i>CryIDa1</i>	<i>CryID</i>	X54160
<i>CryIDb1</i>	<i>PrtB</i>	Z22511
<i>CryIEa1</i>	<i>CryIE</i>	X53985
<i>CryIEa2</i>	<i>CryIE</i>	X56144
<i>CryIEa3</i>	<i>CryIE</i>	M73252
<i>CryIEa4</i>		U94323
<i>CryIEb1</i>	<i>CryIE(b)</i>	M73253
<i>CryIFa1</i>	<i>CryIF</i>	M63897
<i>CryIFa2</i>	<i>CryIF</i>	M63897
<i>CryIFb1</i>	<i>PrtD</i>	Z22512
<i>CryIGa1</i>	<i>PrtA</i>	Z22510
<i>CryIGa2</i>	<i>CryIM</i>	Y09326
<i>CryIGb1</i>	<i>CryH2</i>	
<i>CryIHa1</i>	<i>PrtC</i>	Z22513
<i>CryIHb1</i>		U35780
<i>CryIIa1</i>	<i>CryV</i>	X62821
<i>CryIIa2</i>	<i>CryV</i>	M98544
<i>CryIIa3</i>	<i>CryV</i>	L36338
<i>CryIIa4</i>	<i>CryV</i>	L49391
<i>CryIIa5</i>	<i>CryV</i>	Y08920
<i>CryIIb1</i>	<i>CryV</i>	U07642
<i>CryIIa1</i>	ET4	L32019
<i>CryIIb1</i>	ET1	U31527
<i>CryIKa1</i>		U28801
<i>Cry2Aa1</i>	<i>CryIIA</i>	M31738
<i>Cry2Aa2</i>	<i>CryIIA</i>	M23723
<i>Cry2Aa3</i>		D86084
<i>Cry2Ab1</i>	<i>CryIIB</i>	M23724
<i>Cry2Ab2</i>	<i>CryIIB</i>	X55416
<i>Cry2Ac1</i>	<i>CryIIC</i>	X57252
<i>Cry3Aa1</i>	<i>CryIIIA</i>	M22472
<i>Cry3Aa2</i>	<i>CryIIIA</i>	J02978
<i>Cry3Aa3</i>	<i>CryIIIA</i>	Y00420
<i>Cry3Aa4</i>	<i>CryIIIA</i>	M30503
<i>Cry3Aa5</i>	<i>CryIIIA</i>	M37207
<i>Cry3Aa6</i>	<i>CryIIIA</i>	U10985
<i>Cry3Ba1</i>	<i>CryIIIB</i>	X17123
<i>Cry3Ba2</i>	<i>CryIIIB</i>	A07234
<i>Cry3Bb1</i>	<i>CryIIIB2</i>	M89794
<i>Cry3Bb2</i>	<i>CryIIIC(b)</i>	U31633
<i>Cry3Ca1</i>	<i>CryIIID</i>	X59797
<i>Cry4Aa1</i>	<i>CryIVA</i>	Y00423
<i>Cry4Aa2</i>	<i>CryIVA</i>	D00248
<i>Cry4Ba1</i>	<i>CryIVB</i>	X07423
<i>Cry4Ba2</i>	<i>CryIVB</i>	X07082
<i>Cry4Ba3</i>	<i>CryIVB</i>	M20242
<i>Cry4Ba4</i>	<i>CryIVH</i>	D00247
<i>Cry5Aa1</i>	<i>CryVA(a)</i>	L07025
<i>Cry5Ab1</i>	<i>CryVA(b)</i>	L07026
<i>Cry5Ba1</i>	PS86Q3	U19725
<i>Cry6Aa1</i>	<i>CryVIA</i>	L07022
<i>Cry6Ba1</i>	<i>CryVIB</i>	L07024
<i>Cry7Aa1</i>	<i>CryIIIC</i>	M64478

TABLE 1-continued

KNOWN <i>B. THURINGIENSIS</i> δ -ENDOTOXINS, GENBANK ACCESSION NUMBERS, AND REVISED, NOMENCLATURE ^A		
New	Old	Genbank Accession #
Cry7Ab1	CryIIICb	U04367
Cry8Aa1	CryIIIE	U04364
Cry8Ba1	CryIIIG	U04365
Cry8Ca1	CryIIIF	U04366
Cry9Aa1	CryIG	X58120
Cry9Aa2	CryIG	X58534
Cry9Ba1	CryIX	X75019
Cry9Ca1	CryIH	Z37527
Cry9Da1	N141	D85562
Cry10Aa1	CryIVC	M12662
Cry11Aa1	CryIVD	M31737
Cry11Aa2	CryIVD	M22860
Cry11Ba1	Jeg80	X86902
Cry12Aa1	CryVB	L07027
Cry13Aa1	CryVC	L07023
Cry14Aa1	CryVD	U13955
Cry15Aa1	34kDa	M76442
Cry16Aa1	chm71	X94146
Cry17Ao1	chm71	X99478
Cry18Aa1	CryBP1	X99049
Cry19Aa1	Jeg65	Y08920
Cry20Aa1		U82518
Cry21Aa1		I32932
Cry22Aa1		I34547
Cyt1Aa1	CytA	X03182
Cyt1Aa2	CytA	X04338
Cyt1Aa3	CytA	Y00135
Cyt1Aa4	CytA	M35968
Cyt1Ab1	CytM	X98793
Cyt1Ba1		U37196
Cyt2Aa1	CytB	Z14147
Cyt2Ba1	"CytB"	U52043
Cyt2Ba2	"CytB"	AF020789
Cyt2Ba3	"CytB"	AF022884
Cyt2Ba4	"CytB"	AF022885
Cyt2Ba5	"CytB"	AF022886
Cyt2Bb1		U82519

^AAdapted from: http://epunix.biols.susx.ac.uk/Home/Neil_Grickmore/Bt/index.html

1.2.3 Bioinsecticide Polypeptide Compositions

The utility of bacterial crystal proteins as insecticides was extended beyond lepidopterans and dipteran larvae when the first isolation of a coleopteran-toxic *B. thuringiensis* strain was reported (Krieg et al., 1983; 1984). This strain (described in U.S. Pat. No. 4,766,203, specifically incorporated herein by reference), designated *B. thuringiensis* var. *tenebrionis*, is reported to be toxic to larvae of the coleopteran insects *Agelastica alni* (blue alder leaf beetle) and *Leptinotarsa decemlineata* (Colorado potato beetle).

U.S. Pat. No. 5,024, 837 also describes hybrid *B. thuringiensis* var. *kurstaki* strains which showed activity against lepidopteran insects. U.S. Pat. No. 4,797,279 (corresponding to EP 0221024) discloses a hybrid *B. thuringiensis* containing a plasmid from *B. thuringiensis* var. *kurstaki* encoding a lepidopteran-toxic crystal protein-encoding gene and a plasmid from *B. thuringiensis* *tenebrionis* encoding a coleopteran-toxic crystal protein-encoding gene. The hybrid *B. thuringiensis* strain produces crystal proteins characteristic of those made by both *B. thuringiensis* *kurstaki* and *B. thuringiensis* *tenebrionis*. U.S. Pat. No. 4,910,016 (corresponding to EP 0303379) discloses a *B. thuringiensis* isolate identified as *B. thuringiensis* MT 104 which has insecticidal activity against coleopterans and lepidopterans.

1.2.4 Molecular Genetic Techniques Facilitate Protein Engineering

The revolution in molecular genetics over the past decade has facilitated a logical and orderly approach to engineering

protein with improved properties. Site specific and random mutagenesis methods, the advent of polymerase chain reaction (PCRTM) methodologies, and related advances in the field have permitted an extensive collection of tools for changing both amino acid sequence, and underlying genetic sequences for a variety of proteins of commercial, medical, and agricultural interest.

Following the rapid increase in the number and types of crystal proteins which have been identified in the past decade, researchers began to theorize about using such techniques to improve the insecticidal activity of various crystal proteins. In theory, improvements to δ -endotoxins should be possible using the methods available to protein engineers working in the art, and it was logical to assume that it would be possible to isolate improved variants of the wild-type crystal proteins isolated to date. By strengthening one or more of the aforementioned steps in the mode of action of the toxin, improved molecules should provide enhanced activity, and therefore, represent a breakthrough in the field. If specific amino acid residues on the protein are identified to be responsible for a specific step in the mode of action, then these residues can be targeted for mutagenesis to improve performance.

1.2.5 Structural Analyses of Crystal Proteins

The combination of structural analyses of *B. thuringiensis* toxins followed by an investigation of the function of such structures, motifs, and the like has taught that specific regions of crystal protein endotoxins are, in a general way, responsible for particular functions.

Domain 1, for example, from Cry3Bb and Cry1Ac has been found to be responsible for ion channel activity, the initial step in formation of a pore (Walters et al., 1993; Von Tersch et al., 1994). Domains 2 and 3 have been found to be responsible for receptor binding and insecticidal specificity (Aronson et al., 1995; Caramori et al., 1991; Chen et al., 1993; de Maagd et al., 1996; Ge et al., 1991; Lee et al., 1992; Lee et al., 1995; Lu et al., 1994; Smedley and Ellar, 1996; Smith and Ellar, 1994; Rajamohan et al., 1995; Rajamohan et al., 1996; Wu and Dean, 1996). Regions in domain 2 and 3 can also impact the ion channel activity of some toxins (Chen et al., 1993; Wolfersberger et al., 1996; Von Tersch et al., 1994).

1.3 Deficiencies in the Prior Art

Unfortunately, while many laboratories have attempted to make mutated crystal proteins, few have succeeded in making mutated crystal proteins with improved lepidopteran toxicity. In almost all of the examples of genetically-engineered *B. thuringiensis* toxins in the literature, the biological activity of the mutated crystal protein is no better than that of the wild-type protein, and in many cases, the activity is decreased or destroyed altogether (Almond and Dean, 1993; Aronson et al., 1995; Chen et al., 1993; Chen et al., 1995; Ge et al., 1991; Kwak et al., 1995; Lu et al., 1994; Rajamohan et al., 1995; Rajamohan et al., 1996; Smedley and Ellar, 1996; Smith and Ellar, 1994; Wolfersberger et al., 1996; Wu and Aronson, 1992).

For a crystal protein having approximately 650 amino acids in the sequence of its active toxin, and the possibility of 20 different amino acids at each position in this sequence, the likelihood of arbitrarily creating a successful new structure is remote, even if a general function to a stretch of 250–300 amino acids can be assigned. Indeed, the above prior art with respect to crystal protein gene mutagenesis has been concerned primarily with studying the structure and function of the crystal proteins, using mutagenesis to perturb some step in the mode of action, rather than with engineering improved toxins.

Collectively, the limited successes in the art to develop synthetic toxins with improved insecticidal activity have stifled progress in this area and confounded the search for improved endotoxins or crystal proteins. Rather than following simple and predictable rules, the successful engineering of an improved crystal protein may involve different strategies, depending on the crystal protein being improved and the insect pests being targeted. Thus, the process is highly empirical.

Accordingly, traditional recombinant DNA technology is clearly not routine experimentation for providing improved insecticidal crystal proteins. What are lacking in the prior art are rational methods for producing genetically-engineered *B. thuringiensis* crystal proteins that have improved insecticidal activity and, in particular, improved toxicity towards a wide range of lepidopteran insect pests.

2.0 SUMMARY OF THE INVENTION

The present invention seeks to overcome these and other drawbacks inherent in the prior art by providing genetically-engineered modified *B. thuringiensis* δ -endotoxins (Cry*), and in particular modified Cry3 δ -endotoxins (designated Cry3* endotoxins). Also provided are nucleic acid sequences comprising one or more genes which encode such modified proteins. Particularly preferred genes include cry3* genes such as cry3A*, cry3B*, and cry3C* genes, particularly cry3B* genes, and more particularly, cry3Bb* genes, that encode modified crystal proteins having improved insecticidal activity against target pests.

Also disclosed are novel methods for constructing synthetic Cry3* proteins, synthetically-modified nucleic acid sequences encoding such proteins, and compositions arising therefrom. Also provided are synthetic cry3* expression vectors and various methods of using the improved genes and vectors. In a preferred embodiment, the invention discloses and claims Cry3B* proteins and cry3B* genes which encode improved insecticidal polypeptides.

In preferred embodiments, channel-forming toxin design methods are disclosed which have been used to produce a specific set of designed Cry3Bb* toxins with improved biological activity. These improved Cry3Bb* proteins are listed in Table 2 along with their respective amino acid changes from wild-type (WT) Cry3Bb, the nucleotide changes present in the altered cry3Bb* gene encoding the protein, the fold increase in bioactivity over WT Cry3Bb, the structural site of the alteration, and the design method(s) used to create the new toxins.

Accordingly, the present invention provides in an overall and general sense, mutagenized Cry3 protein-encoding genes and methods of making and using such genes. As used herein the term "mutagenized cry3 gene(s)" means one or more cry3 genes that have been mutagenized or altered to contain one or more nucleotide sequences which are not present in the wild type sequences, and which encode mutant Cry3 crystal proteins (Cry3*) showing improved insecticidal activity. Such mutagenized cry3 genes have been

referred to in the Specification as cry3* genes. Exemplary cry3* genes include cry3A*, cry3B*, and cry3C* genes.

Exemplary mutagenized Cry3 protein-encoding genes include cry3B genes. As used herein the term "mutagenized cry3B gene(s)" means one or more genes that have been mutagenized or altered to contain one or more nucleotide sequences which are not present in the wild type sequences, and which encode mutant Cry3B crystal proteins (Cry3B*) showing improved insecticidal activity. Such genes have been designated cry3B* genes. Exemplary cry3B* genes include cry3Ba* and cry3Bb* genes, which encode Cry3Ba* and Cry3Bb* proteins, respectively.

Likewise, the present invention provides mutagenized Cry3A protein-encoding genes and methods of making and using such genes. As used herein the term "mutagenized cry3A gene(s)" means one or more genes that have been mutagenized or altered to contain one or more nucleotide sequences which are not present in the wild type sequences, and which encode mutant Cry3A crystal proteins (Cry3A*) showing improved insecticidal activity. Such mutagenized genes have been designated as cry3A* genes.

In similar fashion, the present invention provides mutagenized Cry3C protein-encoding genes and methods of making and using such genes. As used herein the term "mutagenized cry3C gene(s)" means one or more genes that have been mutagenized or altered to contain one or more nucleotide sequences which are not present in the wild type sequences, and which encode mutant Cry3C crystal proteins (Cry3C*) showing improved insecticidal activity. Such mutagenized genes have been designated as cry3C* genes.

Preferably the novel sequences comprise nucleic acid sequences in which at least one, and preferably, more than one, and most preferably, a significant number, of wild-type cry3 nucleotides have been replaced with one or more nucleotides, or where one or more nucleotides have been added to or deleted from the native nucleotide sequence for the purpose of altering, adding, or deleting the corresponding amino acids encoded by the nucleic acid sequence so mutagenized. The desired result, therefore, is alteration of the amino acid sequence of the encoded crystal protein to provide toxins having improved or altered activity and/or specificity compared to that of the unmodified crystal protein.

Examples of preferred Cry2Bb*-encoding genes include cry3Bb.60, cry3Bb.11221, cry3Bb.11222, cry3Bb.11223, cry3Bb.11224, cry3Bb.11225, cry3Bb.11226, cry3Bb.11227, cry3Bb.11228, cry3Bb.11229, cry3Bb.11230, cry3Bb.11231, cry3Bb.11232, cry3Bb.11233, cry3Bb.11234, cry3Bb.11235, cry3Bb.11236, cry3Bb.11237, cry3Bb.11238, cry3Bb.11239, cry3Bb.11241, cry3Bb.11242, cry3Bb.11032, cry3Bb.11035, cry3Bb.11036, cry3Bb.11046, cry3Bb.11048, cry3Bb.11051, cry3Bb.11057, cry3Bb.11058, cry3Bb.11081, cry3Bb.11082, cry3Bb.11083, cry3Bb.11084, cry3Bb.11095, and cry3Bb.11098.

TABLE 2

CRY3BB* PROTEINS EXHIBITING IMPROVED ACTIVITY AGAINST SCRW LARVAE						
Cry3Bb* Protein Designation	cry3Bb* Plasmid Designation	cry3Bb* Nucleotide Sequence Changes	Cry3Bb* Amino Acid Changes	Structural Site of Changes	Fold Increase Over WT Activity	Design Method Used
Cry3Bb.60	—	—	Δ 1-159	$\Delta\alpha$ 1- α 3	3.6x	1, 6, 8
Cry3Bb.11221	pEG1707	A460T, C461T, A462T, C464A, T465C, T466C, T467A, A468T, A469T, G470C, T472C, T473G, G474T, A477T, A478T, G479C	T154F, P155H, L156H, L158R	1 α 3, 4	6.4x	1, 8

TABLE 2-continued

CRY3BB* PROTEINS EXHIBITING IMPROVED ACTIVITY AGAINST SCRW LARVAE						
Cry3Bb* Protein Designation	cry3Bb* Plasmid Designation	cry3Bb* Nucleotide Sequence Changes	Cry3Bb* Amino Acid Changes	Structural Site of Changes	Fold Increase Over WT Activity	Design Method Used
Cry3Bb.11222	pEG1708	T687C, T688C, A689T, C691A, A692G	Y230L, H231S	$\alpha 6$	4.0x	3, 7
Cry3Bb.11223	pEG1709	T667C, T687C, T688A, A689G, C691A, A692G	S223P, Y230S	$\alpha 6$	2.8x	3
Cry3Bb.11224	pEG1710	T687C, A692G	H231R	$\alpha 6$	5.0x	7, 8
Cry3Bb.11225	pEG1711	T687C, C691A	H231N, T241S	$\alpha 6$	3.6x	7
Cry3Bb.11226	pEG1712	T687C, C691A, A692C, T693C	H231T	$\alpha 6$	3.0x	7, 8
Cry3Bb.11227	pEG1713	C868A, G869A, G870T	R290N	1 $\alpha 7$, $\beta 1$	1.9x	2, 3, 4, 6
Cry3Bb.11228	pEG1714	C932T, A938C, T942G, G949A, T954C	S311L, N313T, E317K	1 $\beta 1$, $\alpha 8$	4.1x	2, 4
Cry3Bb.11229	pEG1715	T931A, A933C, T942A, T945A, G949A, A953G, T954C	S311T, E317K, Y318C	1 $\beta 1$, $\alpha 8$	2.5x	2, 4
Cry3Bb.11230	pEG1716	T931G, A933C, C934G, T945G, C946T, A947G, G951A, T954C	S311A, L312V, Q316W	1 $\beta 1$, $\alpha 8$	4.7x	2, 4, 8
Cry3Bb.11231	pEG1717	T687C, A692G, C932T, A938C, T942G, G949A, T954C	H231R, S311L, N313T, E317K	$\alpha 6$; 1 $\beta 1$, $\alpha 8$	7.9x	2, 4, 7, 8, 10
Cry3Bb.11232	pEG1718	T931A, A933G, T935C, T936A, A938C, T939C, T942C, T945A, G951T, T954C	S311T, L312P, N313T, E317N	1 $\beta 1$, $\alpha 8$	5.1x	4
Cry3Bb.11233	pEG1719	T931G, A933C, T936G, T942C, C943T, T945A, C946G, G948C, T954C	S311A, Q316D	1 $\beta 1$, $\alpha 8$	2.2x	2, 4
Cry3Bb.11234	pEG1720	T861C, T866C, C868A, T871C, T872G, A875T, T877A, C878G, A882G	I289T, L291R, Y292F, S293R	1 $\alpha 7$, $\beta 1$	4.1x	4
Cry3Bb.11235	pEG1721	T687C, A692G, C932T	H231R, S311L	$\alpha 6$; 1 $\beta 1$, $\alpha 8$	3.2x	2, 4, 7, 8, 10
Cry3Bb.11236	pEG1722	T931A, C932T, A933C, T936C, T942G, T945A, T954C	S311I	1 $\beta 1$, $\alpha 8$	3.1x	2, 4
Cry3Bb.11237	pEG1723	T931A, C932T, A933C, T936C, A937G, A938T, C941A, T942C, T945A, C946A, A947T, A950T, T954C	S311I, N313H	1 $\beta 1$, $\alpha 8$	5.4x	2, 4
Cry3Bb.11238	pEG1724	A933C, T936C, A937G, A938T, C941A, T942C, T945A, C946A, A947T, A950T, T954C	N313V, T314N, Q316M, E317V	1 $\beta 1$, $\alpha 8$	2.6x	2, 4
Cry3Bb.11239	pEG1725	A933T, A938G, T939G, T942A, T944C, T945A, A947T, G948T, A950C, T954C	N313R, L315P, Q316L, E317A	1 $\beta 1$, $\alpha 8$	2.8x	2, 4
Cry3Bb.11241	pEG1726	A860T, T861C, G862A, C868T, G869T, T871C, A873T, T877A, C878G, A879T	Y287F, D288N, R290L	1 $\alpha 7$, $\beta 1$	2.6x	2, 3, 4, 6
Cry3Bb.11242	pEG1727	C868G, G869T	R290V	1 $\alpha 7$, $\beta 1$	2.5x	2, 3, 4, 6, 8
Cry3Bb.11032	pEG1041	A494G	D165G	$\alpha 4$	3.1x	2, 4, 8
Cry3Bb.11035	pEG1046	G479A, A481C, A482C, A484C, G485A, A486C, A494G	S160N, K161P, [P162H] R162H, D165G	$\alpha 4$	2.7x	8
Cry3Bb.11036	pEG1047	A865G, T877C	I289V, S293P	1 $\alpha 7$, $\beta 1$	4.3x	4
Cry3Bb.11046	pEG1052	G479A, A481C, A482C, A484C, G485A, A486C, A494G, A865G, T877C	S160N, K161P, [P162H] R162H, D165G, I289V, S293P	$\alpha 4$; 1 $\alpha 7$, $\beta 1$	2.6x	2, 4, 8, 10
Cry3Bb.11048	pEG1054	T309A, Δ 310, Δ 311, Δ 312	D103E, Δ A104	1 $\alpha 2a$, 2b	4.3x	8
Cry3Bb.11051	pEG1057	A565G, A566G	K189G	1 $\alpha 4$, 5	3.0x	2, 3, 4
Cry3Bb.11057	pEG1062	T309A, Δ 310, Δ 311, Δ 312, G479A, A481C, A482C, A484C, G485A, A486C, A494G	D103E, Δ A104, S160N, K161P, [P162H] R162H, D165G	1 $\alpha 2a$, 2b; $\alpha 4$	3.4x	2, 4, 8, 10
Cry3Bb.11058	pEG1063	T309A, Δ 310, Δ 311, Δ 312, A460T, C461T, A462T, C464A, T465C, T466C, T467A, A468T, A469T, G470C, T472C, T473G, G474T, A477T, A478T, G479C	D103E, Δ A104, T154F, P155H, L156H, L158R	1 $\alpha 2a$, 2b; 1 $\alpha 3$, 4	3.5x	1, 8, 10
Cry3Bb.11081	pEG1084	A494G, T931A, A933C, T942A, T945A, G949A, T954C	D165G, S311T, E317K	$\alpha 4$; 1 $\beta 1$, $\alpha 8$	6.1x	2, 4, 8, 10
Cry3Bb.11082	pEG1085	A494G, A865G, T877C, T914C, T931G, A933C, C934G, T945G, C946T, A947G, G951A, T954C, A1043G, T1094C	D165G, I289V, S293P, P305S, S311A, L312V, Q316W, Q348R, V365A	$\alpha 4$; 1 $\alpha 7$, $\beta 1$; $\beta 1$; 1 $\beta 1$, $\alpha 8$; $\beta 2$; $\beta 3b$	4.9x 9, 10	2, 4, 5, 8
Cry3Bb.11083	pEG1086	A865G, T877C, A1043G	I289V, S293P, Q348R	1 $\alpha 7$, $\beta 1$; $\beta 2$	7.4x	4, 5, 9, 10

TABLE 2-continued

CRY3BB* PROTEINS EXHIBITING IMPROVED ACTIVITY AGAINST SCRW LARVAE						
Cry3Bb* Protein Designation	cry3Bb* Plasmid Designation	cry3Bb* Nucleotide Sequence Changes	Cry3Bb* Amino Acid Changes	Structural Site of Changes	Fold Increase Over WT Activity	Design Method Used
Cry3Bb.11084	pEG1087	A494G, C932T	D165G, S311L	α 4; 1 β 1, α 8	7.2x	2, 4, 8, 10
Cry3Bb.11095	pEG1095	A1043G	Q348R	β 2	4.6x	5, 9
Cry3Bb.11098	pEG1098	A494G, T687C, A692G, C932T, A938C, T942G, G949A, T954C	D165G, H231R, S311L, N313T, E317K	α 4; α 6, 1 β 1, α 8	7.9x	2, 4, 7, 8

In a variety of illustrative embodiments, the inventors have shown remarkable success in generating toxins with improved insecticidal activity using these methods. In particular, the inventors have identified unique methods of analyzing and designing toxins having improved or enhanced insecticidal properties both in vitro and in vivo.

In addition to modifications of Cry3Bb peptides, those having benefit of the present teaching are now also able to make mutations in a variety of channel-forming toxins, and particularly in crystal proteins which are related to Cry3Bb either functionally or structurally. In fact, the inventors contemplate that any *B. thuringiensis* crystal protein or peptide can be analyzed using the methods disclosed herein and may be altered using the methods disclosed herein to produce crystal proteins having improved insecticidal specificity or activity. Alternatively, the inventors contemplate that those of skill in the art having the benefit of the teachings disclosed herein will be able to prepare not only mutated Cry3 toxins with improved activity, but also other crystal proteins including all of those proteins identified in Table 1, herein. In particular, the inventors contemplate the creation of Cry3* variants using one or more of the methods disclosed herein to produce toxins with improved activity. For example, the inventors note Cry3A, Cry3B, and Cry3C crystal proteins (which are known in the art) may be modified using one or more of the design strategies employed herein, to prepare synthetically-modified crystal proteins with improved properties. Likewise, one of skill in the art will even be able to utilize the teachings of the present disclosure to modify other channel forming toxins, including channel forming toxins other than *B. thuringiensis* crystal proteins, and even to modify proteins and channel toxins not yet described or characterized.

Because the structures for insecticidal crystal proteins show a remarkable conservation of protein tertiary structure (Grochulski et al., 1995), and because many crystal proteins show significant amino acid sequence identity to the Cry3Bb amino acid sequence within domain 1, including proteins of the Cry1, Cry2, Cry3, Cry4, Cry5, Cry7, Cry8, Cry9, Cry10, Cry11, Cry12, Cry13, Cry14, and Cry16 classes (Table 1), now in light of the inventors' surprising discovery, for the first time, those of skill in the art having benefit of the teachings disclosed herein will be able to broadly apply the methods of the invention to modifying a host of crystal proteins with improved activity or altered specificity. Such methods will not only be limited to the insecticidal crystal proteins disclosed in Table 1, but may also be applied to any other related crystal protein, including those yet to be identified.

In particular, the high degree of homology between Cry3A, Cry3B, and Cry3C proteins is evident in the alignment of the primary amino acid sequence of the three proteins (FIG. 17A, FIG. 17B, and FIG. 17C).

As such, the disclosed methods may be now applied to preparation of modified crystal proteins having one or more alterations introduced using one or more of the mutational design methods as disclosed herein. The inventors further contemplate that regions may be identified in one or more domains of a crystal protein, or other channel forming toxin which may be similarly modified through site-specific or random mutagenesis to generate toxins having improved activity, or alternatively, altered specificity.

In certain applications, the creation of altered toxins having increased activity against one or more insects is desired. Alternatively, it may be desirable to utilize the methods described herein for creating and identifying altered insecticidal crystal proteins which are active against a wider spectrum of susceptible insects. The inventors further contemplate that the creation of chimeric insecticidal crystal proteins comprising one or more of these mutations may be desirable for preparing "super" toxins which have the combined advantages of increased insecticidal activity and concomitant broad spectrum activity.

In light of the present disclosure, the mutagenesis of one or more codons within the sequence of a toxin may result in the generation of a host of related insecticidal proteins having improved activity. While exemplary mutations have been described for each of the design strategies employed in the present invention, the inventors contemplate that mutations may also be made in insecticidal crystal proteins, including the loop regions, helices regions, active sites of the toxins, regions involved in protein oligomerization, and the like, which will give rise to functional bioinsecticidal crystal proteins. All such mutations are considered to fall within the scope of this disclosure.

In one illustrative embodiment, mutagenized cry3Bb* genes are obtained which encode Cry3Bb* variants that are generally based upon the wild-type Cry3Bb sequence, but that have one or more changes incorporated into the amino acid sequence of the protein using one or more of the design strategies described and claimed herein.

In these and other embodiments, the mutated genes encoding the crystal proteins may be modified so as to change about one, two, three, four, or five or so amino acids in the primary sequence of the encoded polypeptide. Alternatively even more changes from the native sequence may be introduced, such that the encoded protein may have at least about 1% or 2%, or alternatively about 3% or about 4%, or even about 5% to about 10%, or about 10% to about 15%, or even about 15% to about 20% or more of the codons either altered, deleted, or otherwise modified. In certain situations, it may even be desirable to alter substantially more of the primary amino acid sequence to obtain the desired modified protein. In such cases the inventors contemplate that from about 25%, to about 50%, or even from about 50% to about 75%, or more of the native (or wild-

type) codons either altered, deleted, or otherwise modified. Alternatively, mutations in the amino acid sequences or underlying DNA gene sequences which result in the insertion or deletion of one or more amino acids within one or more regions of the crystal protein or peptide.

To effect such changes in the primary sequence of the encoded polypeptides, it may be desirable to mutate or delete one or more nucleotides from the nucleic acid sequences of the genes encoding such polypeptides, or alternatively, under certain circumstances to add one or more nucleotides into the primary nucleic acid sequence at one or more sites in the sequence. Frequently, several nucleotide residues may be altered to produce the desired polypeptide. As such, the inventors contemplate that in certain embodiments it may be desirable to alter only one, two, three, four, or five or so nucleotides in the primary sequence. In other embodiments, which more changes are desired, the mutagenesis may involve changing, deleting, or inserting 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or even 20 or so nucleotide residues in the gene sequence. In still other embodiments, one may desire to mutate, delete, or insert 21, 22, 23, 24, 25, 26, 27, 28, 29, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, or even 90-100, 150, 200, 250, 300, 350, 400, 450, or more nucleotides in the sequence of the gene in order to prepare a cry3* gene which produces a Cry3* polypeptide having the desired characteristics. In fact, any number of mutations, deletions, and/or insertions may be made in the primary sequence of the gene, so long as the encoded protein has the improved insecticidal activity or specificity characteristics described herein.

Changing a large number of the codons in the nucleotide sequence of an endotoxin-encoding gene may be particularly desirable and often necessary to achieve the desired results, particularly in the situation of "plantizing" a DNA sequence in order to express a DNA of non-plant origin in a transformed plant cell. Such methods are routine to those of skill in the plant genetics arts, and frequently many residues of a primary gene sequence will be altered to facilitate expression of the gene in the plant cell. Preferably, the changes in the gene sequence introduce no changes in the amino acid sequence, or introduce only conservative replacements in the amino acid sequence such that the polypeptide produced in the plant cell from the "plantized" nucleotide sequence is still fully functional, and has the desired qualities when expressed in the plant cell.

Genes and encoded proteins mutated in the manner of the invention may also be operatively linked to other protein-encoding nucleic acid sequences, or expressed as fusion proteins. Both N-terminal and C-terminal fusion proteins are contemplated. Virtually any protein- or peptide-encoding DNA sequence, or combinations thereof, may be fused to a mutated cry3* sequence in order to encode a fusion protein. This includes DNA sequences that encode targeting peptides, proteins for recombinant expression, proteins to which one or more targeting peptides is attached, protein subunits, domains from one or more crystal proteins, and the like. Such modifications to primary nucleotide sequences to enhance, target, or optimize expression to the gene sequence in a particular host cell, tissue, or cellular localization, are well-known to those of skill in the art of protein engineering and molecular biology, and it will be readily apparent to such artisans, having benefit of the teachings of this specification, how to facilitate such changes in the nucleotide sequence to produce the polypeptides and polynucleotides disclosed herein.

In one aspect, the invention discloses and claims host cells comprising one or more of the modified crystal proteins

disclosed herein, and in particular, cells of *B. thuringiensis* strains EG11221, EG11222, EG11223, EG11224, EG11225, EG11226, EG11227, EG11228, EG11229, EG11230, EG11231, EG11232, EG11233, EG11234, EG11235, EG11236, EG11237, EG11238, EG11239, EG11241, EG11242, EG11032, EG11035, EG11036, EG11046, EG11048, EG11051, EG11057, EG11058, EG11081, EG11082, EG11083, EG11084, EG11095, and EG11098 which comprise recombinant DNA segments encoding synthetically-modified Cry3Bb* crystal proteins which demonstrates improved insecticidal activity.

Likewise, the invention also discloses and claims cell cultures of *B. thuringiensis* EG11221, EG11222, EG11223, EG11224, EG11225, EG11226, EG11227, EG11228, EG11229, EG11230, EG11231, EG11232, EG11233, EG11234, EG11235, EG11236, EG11237, EG11238, EG11239, EG11241, EG11242, EG11032, EG11035, EG11036, EG11046, EG11048, EG11051, EG11057, EG11058, EG11081, EG11082, EG11083, EG11084, and EG11095, and EG11098.

Such cell cultures may be biologically-pure cultures consisting of a single strain, or alternatively may be cell co-cultures consisting of one or more strains. Such cell cultures may be cultivated under conditions in which one or more additional *B. thuringiensis* or other bacterial strains are simultaneously co-cultured with one or more of the disclosed cultures, or alternatively, one or more of the cell cultures of the present invention may be combined with one or more additional *B. thuringiensis* or other bacterial strains following the independent culture of each. Such procedures may be useful when suspensions of cells containing two or more different crystal proteins are desired.

The subject cultures have been deposited under conditions that assure that access to the cultures will be available during the pendency of this patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 C.F.R. §1.14 and 35 U.S.C. §122. The deposits are available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

Further, the subject culture deposits will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, i.e., they will be stored with all the care necessary to keep them viable and uncontaminated for a period of at least five years after the most recent request for the finishing of a sample of the deposit, and in any case, for a period of at least 30 (thirty) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the cultures. The depositor acknowledges the duty to replace the deposits should the depository be unable to furnish a sample when requested, due to the condition of the deposits. All restrictions on the availability to the public of the subject culture deposits will be irrevocably removed upon the granting of a patent disclosing them.

Cultures shown in Table 3 were deposited in the permanent collection of the Agricultural Research Service Culture Collection, Northern Regional Research Laboratory (NRRL) under the terms of the Budapest Treaty.

TABLE 3

STRAINS OF THE PRESENT INVENTION DEPOSITED UNDER
THE TERMS OF THE BUDAPEST TREATY

Strain	Deposit Date	Protein	Accession Number (NRRL Number)
EG11032	5/27/97	Cry3Bb.11032	B-21744
EG11035	5/27/97	Cry3Bb.11035	B-21745
EG11036	5/27/97	Cry3Bb.11036	B-21746
EG11037	5/27/97	Cry3Bb.11037	B-21747
EG11046	5/27/97	Cry3Bb.11046	B-21748
EG11048	5/27/97	Cry3Bb.11048	B-21749
EG11051	5/27/97	Cry3Bb.11051	B-21750
EG11057	5/27/97	Cry3Bb.11057	B-21751
EG11058	5/27/97	Cry3Bb.11058	B-21752
EG11081	5/27/97	Cry3Bb.11081	B-21753
EG11082	5/27/97	Cry3Bb.11082	B-21754
EG11083	5/27/97	Cry3Bb.11083	B-21755
EG11084	5/27/97	Cry3Bb.11084	B-21756
EG11095	5/27/97	Cry3Bb.11095	B-21757
EG11204	5/27/97	Cry3Bb.11204	B-21758
EG11221	5/27/97	Cry3Bb.11221	B-21759
EG11222	5/27/97	Cry3Bb.11222	B-21760
EG11223	5/27/97	Cry3Bb.11223	B-21761
EG11224	5/27/97	Cry3Bb.11224	B-21762
EG11225	5/27/97	Cry3Bb.11225	B-21763
EG11226	5/27/97	Cry3Bb.11226	B-21764
EG11227	5/27/97	Cry3Bb.11227	B-21765
EG11228	5/27/97	Cry3Bb.11228	B-21766
EG11229	5/27/97	Cry3Bb.11229	B-21767
EG11230	5/27/97	Cry3Bb.11230	B-21768
EG11231	5/27/97	Cry3Bb.11231	B-21769
EG11232	5/27/97	Cry3Bb.11232	B-21770
EG11233	5/27/97	Cry3Bb.11233	B-21771
EG11234	5/27/97	Cry3Bb.11234	B-21772
EG11235	5/27/97	Cry3Bb.11235	B-21773
EG11236	5/27/97	Cry3Bb.11236	B-21774
EG11237	5/27/97	Cry3Bb.11237	B-21775
EG11238	5/27/97	Cry3Bb.11238	B-21776
EG11239	5/27/97	Cry3Bb.11239	B-21777
EG11241	5/27/97	Cry3Bb.11241	B-21778
EG11242	5/27/97	Cry3Bb.11242	B-21779

Also disclosed are methods of controlling or eradicating an insect population from an environment. Such methods generally comprise contacting the insect population to be controlled or eradicated with an insecticidally-effective amount of a Cry3* crystal protein composition. Preferred Cry3* compositions include Cry3A*, Cry3B*, and Cry3C* polypeptide compositions, with Cry3B* compositions being particularly preferred. Examples of such polypeptides include proteins selected from the group consisting of Cry3Bb-60, Cry3Bb.11221, Cry3Bb.11222, Cry3Bb.11223, Cry3Bb.11224, Cry3Bb.11225, Cry3Bb.11226, Cry3Bb.11227, Cry3Bb.11228, Cry3Bb.11229, Cry3Bb.11230, Cry3Bb.11231, Cry3Bb.11232, Cry3Bb.11233, Cry3Bb.11234, Cry3Bb.11235, Cry3Bb.11236, Cry3Bb.11237, Cry3Bb.11238, Cry3Bb.11239, Cry3Bb.11241, Cry3Bb.11242, Cry3Bb.11032, Cry3Bb.11035, Cry3Bb.11036, Cry3Bb.11046, Cry3Bb.11048, Cry3Bb.11051, Cry3Bb.11057, Cry3Bb.11058, Cry3Bb.11081, Cry3Bb.11082, Cry3Bb.11083, Cry3Bb.11084, Cry3Bb.11095, and Cry3Bb.11098.

In preferred embodiments, these Cry3Bb* crystal protein compositions comprise the amino acid sequence of any of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID

NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, SEQ ID NO:102 or SEQ ID NO:108.

2.1 Methods for Producing Modified Cry* Proteins

The modified Cry* polypeptides of the present invention are preparable by a process which generally involves the steps of obtaining a nucleic acid sequence encoding a Cry* polypeptide; analyzing the structure of the polypeptide to identify particular "target" sites for mutagenesis of the underlying gene sequence; introducing one or more mutations into the nucleic acid sequence to produce a change in one or more amino acid residues in the encoded polypeptide sequence; and expressing in a transformed host cell the mutagenized nucleic acid sequence under conditions effective to obtain the modified Cry* protein encoded by the cry* gene.

Means for obtaining the crystal structures of the polypeptides of the invention are well-known. Exemplary high resolution crystal structure solution sets are given in Section 9.0 of the disclosure, and include the crystal structure of both the Cry3A and Cry3B polypeptides disclosed herein. The information provided in Section 9.0 permits the analyses disclosed in each of the methods herein which rely on the 3D crystal structure information for targeting mutagenesis of the polypeptides to particular regions of the primary amino acid sequences of the δ -endotoxins to obtain mutants with increased insecticidal activity or enhanced insecticidal specificity.

A first method for producing a modified *B. thuringiensis* Cry3Bb δ -endotoxin having improved insecticidal activity or specificity disclosed herein generally involves obtaining a high-resolution 3D crystal structure of the endotoxin, locating in the crystal structure one or more regions of bound water wherein the bound water forms a contiguous hydrated surfaces separated by no more than about 16 Å; increasing the number of water molecules in this surface by increasing the hydrophobicity of one or more amino acids of the protein in the region; and obtaining the modified δ -endotoxin so produced. Exemplary δ -endotoxins include Cry3Bb.11032, Cry3Bb.11227, Cry3Bb.11241, Cry3Bb.11051, Cry3Bb.11242, and Cry3Bb.11098.

A second method for producing a modified *B. thuringiensis* Cry3Bb δ -endotoxin having improved insecticidal activity comprises identifying a loop region in a δ -endotoxin; modifying one or more amino acids in the loop to increase the hydrophobicity of the amino acids; and obtaining the modified δ -endotoxin so produced. Preferred δ -endotoxin-produced by this method include Cry3Bb.11241, Cry3Bb.11242, Cry3Bb.11228, Cry3Bb.11229, Cry3Bb.11230, Cry3Bb.11231, Cry3Bb.11233, Cry3Bb.11236, Cry3Bb.11237, Cry3Bb.11238, and Cry3Bb.11239.

A method for increasing the mobility of channel forming helices of a *B. thuringiensis* Cry3B δ -endotoxin is also provided by the present invention. The method generally comprises disrupting one or more hydrogen bonds formed between a first amino acid of one or more of the channel forming helices and a second amino acid of the δ -endotoxin. The hydrogen bonds may be formed inter- or intramolecularly, and the disrupting may consist of replacing a first or second amino acid with a third amino acid whose spatial distance is greater than about 3 Å, or whose spatial orientation bond angle is not equal to 180 ± 60 degrees

relative to the hydrogen bonding site of the first or second amino acid. δ -endotoxins produced by this method and disclosed herein include Cry3Bb.11222, Cry3Bb.11223, Cry3Bb.11224, Cry3Bb.11225, Cry3Bb.11226, Cry3Bb.11227, Cry3Bb.11231, Cry3Bb.11241, and Cry3Bb.11242, and Cry3Bb.11098.

Also disclosed is a method of increasing the flexibility of a loop region in a channel forming domain of a *B. thuringiensis* Cry3Bb δ -endotoxin. This method comprises obtaining a crystal structure of a Cry3Bb δ -endotoxin having one or more loop regions; identifying the amino acids comprising the loop region; and altering one or more of the amino acids to reduce steric hindrance in the loop region, wherein the altering increases flexibility of the loop region in the δ -endotoxin. Examples of δ -endotoxins produced using this method include Cry3Bb.11032, Cry3Bb.11051, Cry3Bb.11228, Cry3Bb.11229, Cry3Bb.11230, Cry3Bb.11231, Cry3Bb.11232, Cry3Bb.11233, Cry3Bb.11236, Cry3Bb.11237, Cry3Bb.11238, Cry3Bb.11239, Cry3Bb.11227, Cry3Bb.11234, Cry3Bb.11241, Cry3Bb.11242, Cry3Bb.11036, and Cry3Bb.11098.

Another aspect of the invention is a method for increasing the activity of a δ -endotoxin, comprising reducing or eliminating binding of the δ -endotoxin to a carbohydrate in a target insect gut. The eliminating or reducing may be accomplished by removal of one or more α helices of domain 1 of the δ -endotoxin, for example, by removal of α helices α 1, α 2a/b, and α 3. An exemplary δ -endotoxin produced using the method is Cry3Bb.60.

Alternatively, the reducing or eliminating may be accomplished by replacing one or more amino acids within loop Pl_o8, with one or more amino acids having increased hydrophobicity. Such a method gives rise to δ -endotoxins such as Cry3Bb.11228, Cry3Bb.11230, Cry3Bb.11231, Cry3Bb.11237, and Cry3Bb.11098, which are described in detail, herein.

Alternatively, the reducing or eliminating is accomplished by replacing one or more specific amino acids, with any other amino acid. Such replacements are described in Table 2, and in the examples herein. One example is the δ -endotoxin designated herein as Cry3Bb.11221.

A method of identifying a region of a Cry3Bb δ -endotoxin for targeted mutagenesis comprising: obtaining a crystal structure of the δ -endotoxin; identifying from the crystal structure one or more surface-exposed amino acids in the protein; randomly substituting one or more of the surface-exposed amino acids to obtain a plurality of mutated polypeptides, wherein at least 50% of the mutated polypeptides have diminished insecticidal activity; and identifying from the plurality of mutated polypeptides one or more regions of the Cry3Bb δ -endotoxin for targeted mutagenesis. The method may further comprise determining the amino acid sequences of a plurality of mutated polypeptides having diminished activity, and identifying one or more amino acid residues required for insecticidal activity.

In another embodiment, the invention provides a process for producing a Cry3Bb δ -endotoxin having improved insecticidal activity. The process generally involves the steps of obtaining a high-resolution crystal structure of the protein; determining the electrostatic surface distribution of the protein; identifying one or more regions of high electrostatic diversity; modifying the electrostatic diversity of the region by altering one or more amino acids in the region; and obtaining a Cry3Bb δ -endotoxin which has improved insecticidal activity. In one embodiment, the electrostatic diver-

sity may be decreased relative to the electrostatic diversity of a native Cry3Bb δ -endotoxin. Exemplary δ -endotoxins with decreased electrostatic diversity include Cry3Bb.11227, Cry3Bb.11241, and Cry3Bb.11242. Alternatively, the electrostatic diversity may be increased relative to the electrostatic diversity of a native Cry3Bb δ -endotoxin. An exemplary δ -endotoxin with increased electrostatic diversity is Cry3Bb.11234.

Furthermore, the invention also provides a method of producing a Cry3Bb δ -endotoxin having improved insecticidal activity which involves obtaining a high-resolution crystal structure; identifying the presence of one or more metal binding sites in the protein; altering one or more amino acids in the binding site; and obtaining an altered protein, wherein the protein has improved insecticidal activity. The altering may involve the elimination of one or more metal binding sites. Exemplary δ -endotoxins include Cry3Bb.11222, Cry3Bb.11224, Cry3Bb.11225, and Cry3Bb.11226.

A further aspect of the invention involves a method of identifying a *B. thuringiensis* Cry3Bb δ -endotoxin having improved channel activity. This method in an overall sense involves obtaining a Cry3Bb δ -endotoxin suspected of having improved channel activity; and determining one or more of the following characteristics in the δ -endotoxin, and comparing such characteristics to those obtained for the wild-type unmodified δ -endotoxin: (1) the rate of channel formation, (2) the rate of growth of channel conductance or (3) the duration of open channel state. From this comparison, one may then select a δ -endotoxin which has an increased rate of channel formation compared to the wild-type δ -endotoxin. Examples of Cry3Bb δ -endotoxins prepared by this method include Cry3Bb.60, Cry3Bb.11035, Cry3Bb.11048, Cry3Bb.11032, Cry3Bb.11223, Cry3Bb.11224, Cry3Bb.11226, Cry3Bb.11221, Cry3Bb.11242, Cry3Bb.11230, and Cry3Bb.11098.

Also provided is a method for producing a modified Cry3Bb δ -endotoxin, having improved insecticidal activity which involves altering one or more non-surface amino acids located at or near the point of greatest convergence of two or more loop regions of the Cry3Bb δ -endotoxin, such that the altering decreases the mobility of one or more of the loop regions. The mobility may conveniently be determined by comparing the thermal denaturation of the modified protein to a wild-type Cry3Bb δ -endotoxin. An exemplary crystal protein produced by this method is Cry3Bb.11095.

A further aspect of the invention involves a method for preparing a modified Cry3Bb δ -endotoxin, having improved insecticidal activity comprising modifying one or more amino acids in the loop to increase the hydrophobicity of said amino acids; and altering one or more of said amino acids to reduce steric hindrance in the loop region, wherein the altering increases flexibility of the loop region in the endotoxin. Exemplary Cry3Bb δ -endotoxins produced is selected from the group consisting of Cry3Bb.11057, Cry3Bb.11058, Cry3Bb.11081, Cry3Bb.11082, Cry3Bb.11083, Cry3Bb.11084, Cry3Bb.11231, Cry3Bb.11235, and Cry3Bb.11098.

The invention also provides a method of improving the insecticidal activity of a *B. thuringiensis* Cry3Bb δ -endotoxin, which generally comprises inserting one or more protease sensitive sites into one or more loop regions of domain 1 of the δ -endotoxin. Preferably, the loop region is α 3,4, and an exemplary δ -endotoxin so produced is Cry3Bb.11221.

2.2 Polypeptide Compositions

The crystal proteins so produced by each of the methods described herein also represent important aspects of the

invention. Such crystal proteins preferably include a protein or peptide selected from the group consisting of Cry3Bb-60, Cry3Bb.11221, Cry3Bb.11222, Cry3Bb.11223, Cry3Bb.11224, Cry3Bb.11225, Cry3Bb.11226, Cry3Bb.11227, Cry3Bb.11228, Cry3Bb.11229, Cry3Bb.11230, Cry3Bb.11231, Cry3Bb.11232, Cry3Bb.11233, Cry3Bb.11234, Cry3Bb.11235, Cry3Bb.11236, Cry3Bb.11237, Cry3Bb.11238, Cry3Bb.11239, Cry3Bb.11241, Cry3Bb.11242, Cry3Bb.11032, Cry3Bb.11035, Cry3Bb.11036, Cry3Bb.11046, Cry3Bb.11048, Cry3Bb.11051, Cry3Bb.11057, Cry3Bb.11058, Cry3Bb.11081, Cry3Bb.11082, Cry3Bb.11083, Cry3Bb.11084, Cry3Bb.11095, and Cry3Bb.11098.

In preferred embodiments, the protein comprises a contiguous amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, SEQ ID NO:102, and SEQ ID NO:108.

Highly preferred are those crystal proteins which are encoded by the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, SEQ ID NO:101; or SEQ ID NO:107, or a nucleic acid sequence which hybridizes to the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, SEQ ID NO:101, or SEQ ID NO:107 under conditions of moderate stringency.

Amino acid, peptide and protein sequences within the scope of the present invention include, and are not limited to the sequences set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, SEQ ID NO:102, and SEQ ID NO:108, and alterations in the amino acid sequences including alterations, deletions, mutations, and homologs.

Compositions which comprise from about 0.5% to about 99% by weight of the crystal protein, or more preferably from about 5% to about 75%, or from about 25% to about 50% by weight of the crystal protein are provided herein. Such compositions may readily be prepared using techniques of protein production and purification well-known to those of skill, and the methods disclosed herein. Such a process for preparing a Cry3Bb* crystal protein generally involves the steps of culturing a host cell which expresses the Cry3Bb* protein (such as a *B. thuringiensis* EG11221, EG11222, EG11223, EG11224, EG11225, EG11226, EG11227, EG11228, EG11229, EG11230, EG11231, EG11232, EG11233, EG11234, EG11235, EG11236, EG11237, EG11238, EG11239, EG11241, EG11242, EG11032, EG11035, EG11036, EG11046, EG11048, EG11051, EG11057, EG11058, EG11081, EG11082, EG11083, EG11084, EG11095, or EG11098 cell) under conditions effective to produce the crystal protein, and then obtaining the crystal protein so produced.

The protein may be present within intact cells, and as such, no subsequent protein isolation or purification steps may be required. Alternatively, the cells may be broken, sonicated, lysed, disrupted, or plasmolyzed to free the crystal protein(s) from the remaining cell debris. In such cases, one may desire to isolate, concentrate, or further purify the resulting crystals containing the proteins prior to use, such as, for example, in the formulation of insecticidal compositions. The composition may ultimately be purified to consist almost entirely of the pure protein, or alternatively, be purified or isolated to a degree such that the composition comprises the crystal protein(s) in an amount of from between about 0.5% and about 99% by weight, or in an amount of from between about 5% and about 95% by weight, or in an amount of from between about 15% and about 85% by weight, or in an amount of from between about 25% and about 75% by weight, or in an amount of from between about 40% and about 60% by weight etc.

2.3 Recombinant Vectors Expressing Cry3* Genes

One important embodiment of the invention is a recombinant vector which comprises a nucleic acid segment encoding one or more of the novel *B. thuringiensis* crystal proteins disclosed herein. Such a vector may be transferred to and replicated in a prokaryotic or eukaryotic host, with bacterial cells being particularly preferred as prokaryotic hosts, and plant cells being particularly preferred as eukaryotic hosts.

In preferred embodiments, the recombinant vector comprises a nucleic acid segment encoding the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, SEQ ID NO:102, or SEQ ID NO:108. Highly preferred nucleic acid segments are those which have the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID

NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, SEQ ID NO:101, or SEQ ID NO:107.

Another important embodiment of the invention is a transformed host cell which expresses one or more of these recombinant vectors. The host cell may be either prokaryotic or eukaryotic, and particularly preferred host cells are those which express the nucleic acid segment(s) comprising the recombinant vector which encode one or more B. thuringiensis crystal protein comprising modified amino acid sequences in one or more loop regions of domain 1, or between α helix 7 of domain 1 and β strand 1 of domain 2. Bacterial cells are particularly preferred as prokaryotic hosts, and plant cells are particularly preferred as eukaryotic hosts.

In an important embodiment, the invention discloses and claims a host cell wherein the modified amino acid sequences comprise one or more loop regions between α helices 1 and 2, α helices 2 and 3, α helices 3 and 4, α helices 4 and 5, α helices 5 and 6 or α helices 6 and 7 of domain 1, or between α helix 7 of domain 1 and β strand 1 of domain 2. A particularly preferred host cell is one that comprises the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, SEQ ID NO:102, or SEQ ID NO:108, and more preferably, one that comprises the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, SEQ ID NO:101, or SEQ ID NO:107.

Bacterial host cells transformed with a nucleic acid segment encoding a modified Cry3Bb crystal protein according to the present invention are disclosed and claimed herein, and in particular, a B. thuringiensis cell having designation EG11221, EG11222, EG11223, EG11224, EG11225, EG11226, EG11227, EG11228, EG11229, EG11230, EG11231, EG11232, EG11233, EG11234, EG11235, EG11236, EG11237, EG11238, EG11239, EG11241, EG11242, EG11032, EG11035, EG11036, EG11046, EG11048, EG11051, EG11057, EG11058, EG11081, EG11082, EG11083, EG11084, EG11095, or EG11098.

In another embodiment, the invention encompasses a method of using a nucleic acid segment of the present invention that encodes a cry3Bb* gene. The method generally comprises the steps of: (a) preparing a recombinant vector in which the cry3Bb* gene is positioned under the control of a promoter; (b) introducing the recombinant vector into a host cell; (c) culturing the host cell under conditions

effective to allow expression of the Cry3Bb* crystal protein encoded by said cry3Bb* gene; and (d) obtaining the expressed Cry3Bb* crystal protein or peptide.

A wide variety of ways are available for introducing a B. thuringiensis gene expressing a toxin into the microorganism host under conditions which allow for stable maintenance and expression of the gene. One can provide for DNA constructs which include the transcriptional and translational regulatory signals for expression of the toxin gene, the toxin gene under their regulatory control and a DNA sequence homologous with a sequence in the host organism, whereby integration will occur, and/or a replication system which is functional in the host, whereby integration or stable maintenance will occur.

The transcriptional initiation signals will include a promoter and a transcriptional initiation start site. In some instances, it may be desirable to provide for regulative expression of the toxin, where expression of the toxin will only occur after release into the environment. This can be achieved with operators or a region binding to an activator or enhancers, which are capable of induction upon a change in the physical or chemical environment of the microorganisms. For example, a temperature sensitive regulatory region may be employed, where the organisms may be grown up in the laboratory without expression of a toxin, but upon release into the environment, expression would begin. Other techniques may employ a specific nutrient medium in the laboratory, which inhibits the expression of the toxin, where the nutrient medium in the environment would allow for expression of the toxin. For translational initiation, a ribosomal binding site and an initiation codon will be present.

Various manipulations may be employed for enhancing the expression of the messenger RNA, particularly by using an active promoter, as well as by employing sequences, which enhance the stability of the messenger RNA. The transcriptional and translational termination region will involve stop codon(s), a terminator region, and optionally, a polyadenylation signal. A hydrophobic "leader" sequence may be employed at the amino terminus of the translated polypeptide sequence in order to promote secretion of the protein across the inner membrane.

In the direction of transcription, namely in the 5' to 3' direction of the coding or sense sequence, the construct will involve the transcriptional regulatory region, if any, and the promoter, where the regulatory region may be either 5' or 3' of the promoter, the ribosomal binding site, the initiation codon, the structural gene having an open reading frame in phase with the initiation codon, the stop codon(s), the polyadenylation signal sequence, if any, and the terminator region. This sequence as a double strand may be used by itself for transformation of a microorganism host, but will usually be included with a DNA sequence involving a marker, where the second DNA sequence may be joined to the toxin expression construct during introduction of the DNA into the host.

By a marker is intended a structural gene which provides for selection of those hosts which have been modified or transformed. The marker will normally provide for selective advantage, for example, providing for biocide resistance, e.g., resistance to antibiotics or heavy metals; complementation, so as to provide prototrophy to an auxotrophic host, or the like. Preferably, complementation is employed, so that the modified host may not only be selected, but may also be competitive in the field. One or more markers may be employed in the development of the constructs, as well as for modifying the host. The organisms

may be further modified by providing for a competitive advantage against other wild-type microorganisms in the field. For example, genes expressing metal chelating agents, e.g., siderophores, may be introduced into the host along with the structural gene expressing the toxin. In this manner, the enhanced expression of a siderophore may provide for a competitive advantage for the toxin-producing host, so that it may effectively compete with the wild-type microorganisms and stably occupy a niche in the environment.

Where no functional replication system is present, the construct will also include a sequence of at least 50 basepairs (bp), preferably at least about 100 bp, more preferably at least about 1000 bp, and usually not more than about 2000 bp of a sequence homologous with a sequence in the host. In this way, the probability of legitimate recombination is enhanced, so that the gene will be integrated into the host and stably maintained by the host. Desirably, the toxin gene will be in close proximity to the gene providing for complementation as well as the gene providing for the competitive advantage. Therefore, in the event that a toxin gene is lost, the resulting organism will be likely to also lost the complementing gene and/or the gene providing for the competitive advantage, so that it will be unable to compete in the environment with the gene retaining the intact construct.

A large number of transcriptional regulatory regions are available from a wide variety of microorganism hosts, such as bacteria, bacteriophage, cyanobacteria, algae, fungi, and the like. Various transcriptional regulatory regions include the regions associated with the trp gene, lac gene, gal gene, the λ_L and λ_R promoters, the tac promoter, the naturally-occurring promoters associated with the δ -endotoxin gene, where functional in the host. See for example, U.S. Pat. Nos. 4,332,898; 4,342,832; and 4,356,270 (each of which is specifically incorporated herein by reference). The termination region may be the termination region normally associated with the transcriptional initiation region or a different transcriptional initiation region, so long as the two regions are compatible and functional in the host.

Where stable episomal maintenance or integration is desired, a plasmid will be employed which has a replication system which is functional in the host. The replication system may be derived from the chromosome, an episomal element normally present in the host or a different host, or a replication system from a virus which is stable in the host. A large number of plasmids are available, such as pBR322, pACYC184, RSF1010, pR01614, and the like. See for example, Olson et al. (1982); Bagdasarian et al. (1981), Baum et al., 1990, and U.S. Pat. Nos. 4,356,270; 4,362,817; 4,371,625, and 5,441,884, each incorporated specifically herein by reference.

The *B. thuringiensis* gene can be introduced between the transcriptional and translational initiation region and the transcriptional and translational termination region, so as to be under the regulatory control of the initiation region. This construct will be included in a plasmid, which will include at least one replication system, but may include more than one, where one replication system is employed for cloning during the development of the plasmid and the second replication system is necessary for functioning in the ultimate host. In addition, one or more markers may be present, which have been described previously. Where integration is desired, the plasmid will desirably include a sequence homologous with the host genome.

The transformants can be isolated in accordance with conventional ways, usually employing a selection technique, which allows for selection of the desired organism as against

unmodified organisms or transferring organisms, when present. The transformants then can be tested for pesticidal activity. If desired, unwanted or ancillary DNA sequences may be selectively removed from the recombinant bacterium by employing site-specific recombination systems, such as those described in U.S. Pat. No. 5,441,884 (specifically incorporated herein by reference).

2.4 Cry3 DNA Segments

A *B. thuringiensis* cry3* gene encoding a crystal protein having one or more mutations in one or more regions of the peptide represents an important aspect of the invention. Preferably, the cry3* gene encodes an amino acid sequence in which one or more amino acid residues have been changed based on the methods disclosed herein, and particularly those changes which have been made for the purpose of altering the insecticidal activity or specificity of the crystal protein.

In accordance with the present invention, nucleic acid sequences include and are not limited to DNA, including and not limited to cDNA and genomic DNA, genes; RNA, including and not limited to mRNA and tRNA; antisense sequences, nucleosides, and suitable nucleic acid sequences such as those set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, SEQ ID NO:101, or SEQ ID NO:107, and alterations in the nucleic acid sequences including alterations, deletions, mutations, and homologs capable of expressing the *B. thuringiensis* modified toxins of the present invention.

As such the present invention also concerns DNA segments, that are free from total genomic DNA and that encode the novel synthetically-modified crystal proteins disclosed herein. DNA segments encoding these peptide species may prove to encode proteins, polypeptides, subunits, functional domains, and the like of crystal protein-related or other non-related gene products. In addition these DNA segments may be synthesized entirely in vitro using methods that are well-known to those of skill in the art.

As used herein, the term "DNA segment" refers to a DNA molecule that has been isolated free of total genomic DNA of a particular species. Therefore, a DNA segment encoding a crystal protein or peptide refers to a DNA segment that contains crystal protein coding sequences yet is isolated away from, or purified free from, total genomic DNA of the species from which the DNA segment is obtained, which in the instant case is the genome of the Gram-positive bacterial genus, *Bacillus*, and in particular, the species of *Bacillus* known as *B. thuringiensis*. Included within the term "DNA segment", are DNA segments and smaller fragments of such segments, and also recombinant vectors, including, for example, plasmids, cosmids, phagemids, phage, viruses, and the like.

Similarly, a DNA segment comprising an isolated or purified crystal protein-encoding gene refers to a DNA segment which may include in addition to peptide encoding sequences, certain other elements such as, regulatory sequences, isolated substantially away from other naturally

occurring genes or protein-encoding sequences. In this respect, the term "gene" is used for simplicity to refer to a functional protein-, polypeptide- or peptide-encoding unit. As will be understood by those in the art, this functional unit includes both genomic sequences, operon sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides or peptides.

"Isolated substantially away from other coding sequences" means that the gene of interest, in this case, a gene encoding a bacterial crystal protein, forms the significant part of the coding region of the DNA segment, and that the DNA segment does not contain large portions of naturally-occurring coding DNA, such as large chromosomal fragments or other functional genes or operon coding regions. Of course, this refers to the DNA segment as originally isolated, and does not exclude genes, recombinant genes, synthetic linkers, or coding regions later added to the segment by the hand of man.

Particularly preferred DNA sequences are those encoding Cry3Bb.60, Cry3Bb.11221, Cry3Bb.11222, Cry3Bb.11223, Cry3Bb.11224, Cry3Bb.11225, Cry3Bb.11226, Cry3Bb.11227, Cry3Bb.11228, Cry3Bb.11229, Cry3Bb.11230, Cry3Bb.11231, Cry3Bb.11232, Cry3Bb.11233, Cry3Bb.11234, Cry3Bb.11235, Cry3Bb.11236, Cry3Bb.11237, Cry3Bb.11238, Cry3Bb.11239, Cry3Bb.11241, Cry3Bb.11242, Cry3Bb.11032, Cry3Bb.11035, Cry3Bb.11036, Cry3Bb.11046, Cry3Bb.11048, Cry3Bb.11051, Cry3Bb.11057, Cry3Bb.11058, Cry3Bb.11081, Cry3Bb.11082, Cry3Bb.11083, Cry3Bb.11084, Cry3Bb.11095 and Cry3Bb.11098 crystal proteins, and in particular cry3Bb* genes such as cry3Bb.60, cry3Bb.11221, cry3Bb.11222, cry3Bb.11223, cry3Bb.11224, cry3Bb.11225, cry3Bb.11226, cry3Bb.11227, cry3Bb.11228, cry3Bb.11229, cry3Bb.11230, cry3Bb.11231, cry3Bb.11232, cry3Bb.11233, cry3Bb.11234, cry3Bb.11235, cry3Bb.11236, cry3Bb.11237, cry3Bb.11238, cry3Bb.11239, cry3Bb.11241, cry3Bb.11242, cry3Bb.11032, cry3Bb.11035, cry3Bb.11036, cry3Bb.11046, cry3Bb.11048, cry3Bb.11051, cry3Bb.11057, cry3Bb.11058, cry3Bb.11081, cry3Bb.11082, cry3Bb.11083, cry3Bb.11084, cry3Bb.11095 and cry3Bb.11098. In particular embodiments, the invention concerns isolated DNA segments and recombinant vectors incorporating DNA sequences that encode a Cry peptide species that includes within its amino acid sequence an amino acid sequence essentially as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, SEQ ID NO:102, or SEQ ID NO:108.

The term "a sequence essentially as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID

NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, SEQ ID NO:102, or SEQ ID NO:108" means that the sequence substantially corresponds to a portion of the sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, SEQ ID NO:102, or SEQ ID NO:108, and has relatively few amino acids that are not identical to, or a biologically functional equivalent of, the amino acids of any of these sequences. The term "biologically functional equivalent" is well understood in the art and is further defined in detail herein (e.g., see Illustrative Embodiments).

Accordingly, sequences that have between about 70% and about 75% or between about 75% and about 80%, or more preferably between about 81% and about 90%, or even more preferably between about 91% or 92% or 93% and about 97% or 98% or 99% amino acid sequence identity or functional equivalence to the amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, SEQ ID NO:102 or SEQ ID NO:108 will be sequences that are "essentially as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, SEQ ID NO:102, or SEQ ID NO:108."

It will be understood that amino acid and nucleic acid sequences may include additional residues, such as additional N- or C-terminal amino acids or 5' or 3' sequences, and yet still be essentially as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the maintenance of biological protein activity where protein expression is concerned. The addition of terminal sequences particularly applies to nucleic acid sequences that may, for example, include various non-coding sequences flanking either of the 5' or 3' portions of the coding region or may include various internal sequences, i.e., introns, which are known to occur within genes.

The nucleic acid segments of the present invention, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters,

polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol.

For example, nucleic acid fragments may be prepared that include a short contiguous stretch encoding the peptide sequence disclosed in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, SEQ ID NO:102, or SEQ ID NO:108, or that are identical to or complementary to DNA sequences which encode the peptide disclosed in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, SEQ ID NO:102, or SEQ ID NO:108, and particularly the DNA segments disclosed in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, SEQ ID NO:101, or SEQ ID NO:107.

Highly preferred nucleic acid segments of the present invention comprise one or more cry genes of the invention, or a portion of one or more cry genes of the invention. For certain application, relatively small contiguous nucleic acid sequences are preferable, such as those which are about 14 or 15 or 16 or 17 or 18 or 19, or 20, or 30–50, 51–80, 81–100 or so nucleotides in length. Alternatively, in some embodiments, and particularly those involving preparation of recombinant vectors, transformation of suitable host cells, and preparation of transgenic plant cell, longer nucleic acid segments are preferred, particularly those that include the entire coding region of one or more cry genes: As such, the preferred segments may include those that are up to about 20,000 or so nucleotides in length, or alternatively, shorter sequences such as those about 19,000, about 18,000, about 17,000, about 16,000, about 15,000, about 14,000, about 13,000, about 12,000, 11,000, about 10,000, about 9,000, about 8,000, about 7,000, about 6,000, about 5,000, about 4,500, about 4,000, about 3,500, about 3,000, about 2,500, about 2,000, about 1,500, about 1,000, about 500, or about 200 or so base pairs in length. Of course, these numbers are not intended to be exclusionary of all possible intermediate

lengths in the range of from about 20,000 to about 15 nucleotides, as all of these intermediate lengths are also contemplated to be useful, and fall within the scope of the present invention. It will be readily understood that “intermediate lengths”, in these contexts, means any length between the quoted ranges, such as 14, 15, 16, 17, 18, 19, 20, etc.; 21, 22, 23, 24, 25, 26, 27, 28, 29, etc.; 30, 31, 32, 33, 34, 35, 36 . . . etc.; 40, 41, 42, 43, 44 . . . etc.; 50, 51, 52, 53 . . . etc.; 60, 61, 62, 63 . . . etc.; 70, 80, 90, 100, 110, 120, 130 . . . etc.; 200, 210, 220, 230, 240, 250 . . . etc.; including all integers in the entire range from about 14 to about 10,000, including those integers in the ranges 200–500; 500–1,000; 1,000–2,000; 2,000–3,000; 3,000–5,000 and the like.

In a preferred embodiment, the nucleic acid segments comprise a sequence of from about 1800 to about 18,000 base pair in length, and comprise one or more genes which encode a modified Cry3* polypeptide disclosed herein which has increased activity against Coleopteran insect pests.

It will also be understood that this invention is not limited to the particular nucleic acid sequences which encode peptides of the present invention, or which encode the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, SEQ ID NO:102, or SEQ ID NO:108, including the DNA sequences which are particularly disclosed in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, SEQ ID NO:101, or SEQ ID NO:107. Recombinant vectors and isolated DNA segments may therefore variously include the peptide-coding regions themselves, coding regions bearing selected alterations or modifications in the basic coding region, or they may encode larger polypeptides that nevertheless include these peptide-coding regions or may encode biologically functional equivalent proteins or peptides that have variant amino acids sequences.

The DNA segments of the present invention encompass biologically-functional, equivalent peptides. Such sequences may arise as a consequence of codon redundancy and functional equivalency that are known to occur naturally within nucleic acid sequences and the proteins thus encoded. Alternatively, functionally-equivalent proteins or peptides may be created via the application of recombinant DNA technology, in which changes in the protein structure may be engineered, based on considerations of the properties of the amino acids being exchanged. Changes designed by man may be introduced through the application of site-directed mutagenesis techniques, e.g., to introduce improvements to the antigenicity of the protein or to test mutants in order to examine activity at the molecular level

If desired, one may also prepare fusion proteins and peptides, e.g., where the peptide-coding regions are aligned within the same expression unit with other proteins or peptides having desired functions, such as for purification or immunodetection purposes (e.g., proteins that may be purified by affinity chromatography and enzyme label coding regions, respectively).

Recombinant vectors from further aspects of the present invention. Particularly useful vectors are contemplated to be those vectors in which the coding portion of the DNA segment, whether encoding a full length protein or smaller peptide, is positioned under the control of a promoter. The promoter may be in the form of the promoter that is naturally associated with a gene encoding peptides of the present invention, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment or exon, for example, using recombinant cloning and/or PCR™ technology, in connection with the compositions disclosed herein.

2.5 Vectors, Host Cells, and Protein Expression

In other embodiments, it is contemplated that certain advantages will be gained by positioning the coding DNA segment under the control of a recombinant, or heterologous, promoter. As used herein, a recombinant or heterologous promoter is intended to refer to a promoter that is not normally associated with a DNA segment encoding a crystal protein or peptide in its natural environment. Such promoters may include promoters normally associated with other genes, and/or promoters isolated from any bacterial, viral, eukaryotic, or plant cell. Naturally, it will be important to employ a promoter that effectively directs the expression of the DNA segment in the cell type, organism, or even animal, chosen for expression. The use of promoter and cell type combinations for protein expression is generally known to those of skill in the art of molecular biology, for example, see Sambrook et al., 1989. The promoters employed may be constitutive, or inducible, and can be used under the appropriate conditions to direct high level expression of the introduced DNA segment, such as is advantageous in the large-scale production of recombinant proteins or peptides. Appropriate promoter systems contemplated for use in high-level expression include, but are not limited to, the Pichia expression vector system (Pharmacia LKB Biotechnology).

In connection with expression embodiments to prepare recombinant proteins and peptides, it is contemplated that longer DNA segments will most often be used, with DNA segments encoding the entire peptide sequence being most preferred. However, it will be appreciated that the use of shorter DNA segments to direct the expression of crystal peptides or epitopic core regions, such as may be used to generate anti-crystal protein antibodies, also falls within the scope of the invention. DNA segments that encode peptide antigens from about 8, 9, 10, or 11 or so amino acids, and up to and including those of about 30, 40, or 50 or so amino acids in length, or more preferably, from about 8 to about 30 amino acids in length, or even more preferably, from about 8 to about 20 amino acids in length are contemplated to be particularly useful. Such peptide epitopes may be amino acid sequences which comprise contiguous amino acid sequence from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48,

SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, SEQ ID NO:102, or SEQ ID NO:108.

2.6 Transformed Host Cells and Transgenic Plants

In one embodiment, the invention provides a transgenic plant having incorporated into its genome a transgene that encodes a contiguous amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, SEQ ID NO:102, and SEQ ID NO:108.

A further aspect of the invention is a transgenic plant having incorporated into its genome a cry3Bb* transgene, provided the transgene comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, SEQ ID NO:101, and SEQ ID NO:107. Also disclosed and claimed are progeny of such a transgenic plant, as well as its seed, progeny from such seeds, and seeds arising from the second and subsequent generation plants derived from such a transgenic plant.

The invention also discloses and claims host cells, both native, and genetically engineered, which express the novel cry3Bb* genes to produce Cry3Bb* polypeptides. Preferred examples of bacterial host cells include *B. thuringiensis* EG11221, EG11222, EG11223, EG11224, EG11225, EG11226, EG11227, EG11228, EG11229, EG11230, EG11231, EG11232, EG11233, EG11234, EG11235, EG11236, EG11237, EG11238, EG11239, EG11241, EG11242, EG11032, EG11035, EG11036, EG11046, EG11048, EG11051, EG11057, EG11058, EG11081, EG11082, EG11083, EG11084, EG11095, and EG11098.

Methods of using such cells to produce Cry3* crystal proteins are also disclosed. Such methods generally involve culturing the host cell (such as *B. thuringiensis* EG11221, EG11222, EG11223, EG11224, EG11225, EG11226, EG11227, EG11228, EG11229, EG11230, EG11231, EG11232, EG11233, EG11234, EG11235, EG11236, EG11237, EG11238, EG11239, EG11241, EG11242, EG11032, EG11035, EG11036, EG11046, EG11048, EG11051, EG11057, EG11058, EG11081, EG11082, EG11083, EG11084, or EG11095, or EG 11098) under conditions effective to produce a Cry3* crystal protein, and obtaining the Cry3* crystal protein from said cell.

In yet another aspect, the present invention provides methods for producing a transgenic plant which expresses a

nucleic acid segment encoding the novel recombinant crystal proteins of the present invention. The process of producing transgenic plants is well-known in the art. In general, the method comprises transforming a suitable host cell with one or more DNA segments which contain one or more promoters operatively linked to a coding region that encodes one or more of the disclosed *B. thuringiensis* crystal proteins. Such a coding region is generally operatively linked to a transcription-terminating region, whereby the promoter is capable of driving the transcription of the coding region in the cell, and hence providing the cell the ability to produce the recombinant protein in vivo. Alternatively, in instances where it is desirable to control, regulate, or decrease the amount of a particular recombinant crystal protein expressed in a particular transgenic cell, the invention also provides for the expression of crystal protein antisense mRNA. The use of antisense mRNA as a means of controlling or decreasing the amount of a given protein of interest in a cell is well-known in the art.

Another aspect of the invention comprises a transgenic plant which express a gene or gene segment encoding one or more of the novel polypeptide compositions disclosed herein. As used herein, the term "transgenic plant" is intended to refer to a plant that has incorporated DNA sequences, including but not limited to genes which are perhaps not normally present, DNA sequences not normally transcribed into RNA or translated into a protein ("expressed"), or any other genes or DNA sequences which one desires to introduce into the non-transformed plant, such as genes which may normally be present in the non-transformed plant but which one desires to either genetically engineer or to have altered expression.

It is contemplated that in some instances the genome of a transgenic plant of the present invention will have been augmented through the stable introduction of one or more Cry3Bb*-encoding transgenes, either native, synthetically modified, or mutated. In some instances, more than one transgene will be incorporated into the genome of the transformed host plant cell. Such is the case when more than one crystal protein-encoding DNA segment is incorporated into the genome of such a plant. In certain situations, it may be desirable to have one, two, three, four, or even more *B. thuringiensis* crystal proteins (either native or recombinantly-engineered) incorporated and stably expressed in the transformed transgenic plant.

A preferred gene which may be introduced includes, for example, a crystal protein-encoding a DNA sequence from bacterial origin, and particularly one or more of those described herein which are obtained from *Bacillus* spp. Highly preferred nucleic acid sequences are those obtained from *B. thuringiensis*, or any of those sequences which have been genetically engineered to decrease or increase the insecticidal activity of the crystal protein in such a transformed host cell.

Means for transforming a plant cell and the preparation of a transgenic cell line are well-known in the art, and are discussed herein. Vectors, plasmids, cosmids, YACs (yeast artificial chromosomes) and DNA segments for use in transforming such cells will, of course, generally comprise either the operons, genes, or gene-derived sequences of the present invention, either native, or synthetically-derived, and particularly those encoding the disclosed crystal proteins. These DNA constructs can further include structures such as promoters, enhancers, polylinkers, or even gene sequences which have positively- or negatively-regulating activity upon the particular genes of interest as desired. The DNA segment or gene may encode either a native or modified crystal protein, which will be expressed in the resultant recombinant cells, and/or which will impart an improved phenotype to the regenerated plant

Such transgenic plants may be desirable for increasing the insecticidal resistance of a monocotyledonous or dicotyledonous plant, by incorporating into such a plant, a transgenic DNA segment encoding a Cry3Bb* crystal protein which is toxic to coleopteran insects. Particularly preferred plants include grains such as corn, wheat, rye, rice, barley, and oats; legumes such as soybeans; tubers such as potatoes; fiber crops such as flax and cotton; turf and pasture grasses; ornamental plants; shrubs; trees; vegetables, berries, citrus, fruits, cacti, succulents, and other commercially-important crops including garden and houseplants.

In a related aspect, the present invention also encompasses a seed produced by the transformed plant, a progeny from such seed, and a seed produced by the progeny of the original transgenic plant, produced in accordance with the above process. Such progeny and seeds will have one or more crystal protein transgene(s) stably incorporated into its genome, and such progeny plants will inherit the traits afforded by the introduction of a stable transgene in Mendelian fashion. All such transgenic plants having incorporated into their genome transgenic DNA segments encoding one or more Cry3Bb* crystal proteins or polypeptides are aspects of this invention. Particularly preferred transgenes for the practice of the invention include nucleic acid segments comprising one or more cry3Bb* gene(s).

2.7 Biological Functional Equivalents

Modification and changes may be made in the structure of the peptides of the present invention and DNA segments which encode them and still obtain a functional molecule that encodes a protein or peptide with desirable characteristics. The following is a discussion based upon changing the amino acids of a protein to create an equivalent, or even an improved, second-generation molecule. In particular embodiments of the invention, mutated crystal proteins are contemplated to be useful for increasing the insecticidal activity of the protein, and consequently increasing the insecticidal activity and/or expression of the recombinant transgene in a plant cell. The amino acid changes may be achieved by changing the codons of the DNA sequence, according to the codons given in Table 4.

TABLE 4

Amino Acids		Codons	
Alanine	Ala	A	GCA GCC GCG GGU
Cysteine	Cys	C	UGC UGU
Aspartic Acid	Asp	D	GAC GAU
Glutamic Acid	Glu	B	GAA GAG
Phenylalanine	Phe	P	UUC UUU
Glycine	Gly	G	GGA GGC GGG GGU
Histidine	His	H	CAC CAU
Isoleucine	Ile	I	AUA AUC AUU
Lysine	Lys	K	AAA AAG
Leucine	Leu	L	UUA UUG CUA CUG CUU
Methionine	Met	M	AUG
Asparagine	Asn	N	AAC AAU
Proline	Pro	P	CCA CCC CCG CCU
Glutamine	Gln	Q	CAA CAG
Arginine	Arg	R	AGA AGG CGA CGC CGG CGU
Serine	Ser	S	AGC AGU UCA UCC UCG UCU
Threonine	Thr	T	ACA ACC ACG ACU
Valine	Val	V	GUA GUC GUG GUU
Tryptophan	Trp	W	UGG
Tyrosine	Tyr	Y	UAC UAU

For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's

biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated by the inventors that various changes may be made in the peptide sequences of the disclosed compositions, or corresponding DNA sequences which encode said peptides without appreciable loss of their biological utility or activity.

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, incorporate herein by reference). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics (Kyte and Doolittle, 1982), these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, i.e., still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Pat. No. 4,554,101, specifically incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U.S. Pat. No. 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 $\pm$ 1); glutamate (+3.0 $\pm$ 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 $\pm$ 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4).

It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art

and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

3.0 BRIEF DESCRIPTION OF THE DRAWINGS

The drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented here.

FIG. 1. Schematic representation of the monomeric structure of Cry3Bb.

FIG. 2. Stereoscopic view of the monomeric structure of Cry3Bb with associated water molecules (represented by dots).

FIG. 3A. Schematic representation of domain 1 of Cry3Bb

FIG. 3B. Diagram of the positions of the 7 helices that comprise domain 1.

FIG. 4. Domain 1 of Cry3Bb is organized into seven α helices illustrated in FIG. 3A (schematic representation) and FIG. 3B (schematic diagram). The α helices and amino acids residues are shown.

FIG. 5A. Schematic representation of domain 2 and Cry3Bb.

FIG. 5B. Diagram of the positions of the 11 β strands that compose the 3 β sheets of domain 2.

FIG. 6. Domain 2 of Cry3Bb is a collection of three anti-parallel β sheets illustrated in FIG. 5. The amino acids that define these sheets is listed below ($\alpha 8$, amino acids 322-328, also is included in domain 2):

FIG. 7A. Schematic representation of domain 3 of Cry3Bb.

FIG. 7B. Diagram of the positions of the β strands that comprise domain 3.

FIG. 8. Domain 3 (FIG. 7) is a loosely organized collection of β strands and loops; no β sheets are present. The β strands contain the amino acids limited below:

FIG. 9A. A "side" view of the dimeric structure of Cry3Bb. The helical bundles of domains 1 can be seen in the middle of the molecule.

FIG. 9B. A "top" view of the dimeric structure of Cry3Bb. The helical bundles of domains 1 can be seen in the middle of the molecule.

FIG. 10. A graphic representation of the growth in conductance with time of channels formed by Cry3A and Cry3Bb in planar lipid bilayers. Cry3A forms channels with higher conductances much more rapidly than Cry3Bb.

FIG. 11. A map of pEG1701 which contains the Cry3Bb gene with the cry1F terminator.

FIG. 12. The results of replicated 1-dose assays against SCRW larvae of Cry3Bb proteins altered in the 1B2,3 region.

FIG. 13. The results of replicated, 1-dose assays against SCRW larvae of Cry3Bb proteins altered in the 1B6, 7 region.

FIG. 14. The results of replicated, 1-dose screens against SCRW larvae of Cry3Bb proteins altered in the 1B10,11 region.

FIG. 15. Single channel recordings of channels formed by Cry3Bb.11230 and WT Cry3Bb in planar lipid bilayers. Cry3Bb. 11230 forms channels with well resolved open and closed states while Cry3Bb rarely does.

FIG. 16. Single channel recordings of channels formed by Cry3Bb and Cry3Bb.60, a truncated form of Cry3Bb. Cry3Bb.60 forms channels more quickly than Cry3Bb and, unlike Cry3Bb, produces channels with well resolved open and closed states.

FIG. 17A. Sequence alignment of the amino acid sequence of Cry3A, Cry3B, and Cry3C.

FIG. 17B. Shown is a continuation of alignment of the amino acid sequence of Cry3A, Cry3B, and Cry3C shown in FIG. 17A.

FIG. 17C. Shown is a continuation of alignment of the amino acid sequence of Cry3A, Cry3B, and Cry3C shown in FIG. 17A.

4.0 DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The invention defines new *B. thuringiensis* (Bt) insecticidal δ -endotoxin proteins and the biochemical and biophysical strategies used to design the new proteins. Delta-endotoxins are a class of insecticidal proteins produced by *B. thuringiensis* that form cation-selective channels in planar lipid bilayers (English and Slatin, 1992). The new δ -endotoxins are based on the parent structure of the coleopteran-active, δ -endotoxin Cry3Bb. Like other members of the coleopteran-active class of δ -endotoxins, including Cry3A and Cry3B, Cry3Bb exhibits excellent insecticidal activity against the Colorado Potato Beetle (*Leptinotarsa decemlineata*). However, unlike Cry3A and Cry3B, Cry3Bb is also active against the southern corn rootworm or SCRW (*Diabrotica undecimpunctata howardi* Barber) and the western corn rootworm or WCRW (*Diabrotica virgifera virgifera* LeConte). The new insecticidal proteins described herein were specifically designed to improve the biological activity of the parent Cry3Bb protein. In addition, the design strategies themselves are novel inventions capable of being applied to and improving *B. thuringiensis* δ -endotoxins in general. *B. thuringiensis* δ -endotoxins are also members of a larger class of bacterial toxins that form ion channels (see English and Slatin 1992, for a review). The inventors, therefore, believe that these design strategies can also be applied to any biologically active, channel-forming protein to improve its biological properties.

The designed Cry3Bb proteins were engineered using one or more of the following strategies including (1) identification and alteration of protease-sensitive sites and proteolytic processing; (2) analysis and manipulation of bound water; (3) manipulation of hydrogen bonds around mobile regions; (4) loop analysis and loop redesign around flexible helices; (5) loop design around β strands and β sheets; (6) identification and redesign of complex electrostatic surfaces; (7) identification and removal of metal binding sites; (8) alteration of quaternary structure; (9) identification and design of structural residues; and (10) combinations of any and all sites defined by strategies 1-9. These design strategies permit the identification and redesign of specific sites on Cry3Bb, ultimately creating new proteins with improved insecticidal activities. These new proteins are designated Cry3Bb designed proteins and are named Cry3Bb followed by a period and a suffix (e.g., Cry3Bb.60, Cry3Bb.11231). The new proteins are listed in Table 2 along with the specific sites on the molecule that were modified, the amino-acid sequence changes at those sites that improve biological activity, the improved insecticidal activities and the design method used to identify that specific site.

4.1 Some Advantages of the Invention

Mutagenesis studies with cry genes have failed to identify a significant number of mutant crystal proteins which have

improved broad-spectrum insecticidal activity, that is, with improved toxicity towards a range of insect pest species. Since agricultural crops are typically threatened by more than one insect pest species at any given time, desirable mutant crystal proteins are preferably those that exhibit improvements in toxicity towards multiple insect pest species. Previous failures to identify such mutants may be attributed to the choice of sites targeted for mutagenesis. For example, with respect to the related protein, Cry1C, sites within domain 2 and domain 3 have been the principal targets of mutagenesis efforts, primarily because these domains are believed to be important for receptor binding and in determining insecticidal specificity (Aronson et al., 1995; Chen et al. 1993; de Maagd et al., 1996; Lee et al., 1992; Lee et al., 1995; Lu et al., 1994; Smedley and Ellar, 1996; Smith and Ellar, 1994; Rajamohan et al., 1995; Rajamohan et al., 1996).

In contrast, the present inventors reasoned that the toxicity of Cry3 proteins, and specifically the toxicity of the Cry3Bb protein, may be improved against a broader array of target pests by targeting regions involved in ion channel function rather than regions of the molecule directly involved in receptor interactions, namely domains 2 and 3. Accordingly, the inventors opted to target regions within domain 1 of Cry3Bb for mutagenesis for the purpose of isolating Cry3Bb mutants with improved broad spectrum toxicity. Indeed, in the present invention, Cry3Bb mutants are described that show improved toxicity towards several coleopteran pests.

At least one, and probably more than one, α helix of domain 1 is involved in the formation of ion channels and pores within the insect midgut epithelium (Gazit and Shai, 1993; Gazit and Shai, 1995). Rather than target for mutagenesis the sequences encoding the α helices of domain 1 as others have (Wu and Aronson, 1992; Aronson et al., 1995; Chen et al., 1995), the present inventors opted to target exclusively sequences encoding amino acid residues adjacent to or lying within the predicted loop regions of Cry3Bb that separate these α helices. Amino acid residues within these loop regions or amino acid residues capping the end of an α helix and lying adjacent to these loop regions may affect the spatial relationships among these α helices. Consequently, the substitution of these amino acid residues may result in subtle changes in tertiary structure, or even quaternary structure, that positively impact the function of the ion channel. Amino acid residues in the loop regions of domain 1 are exposed to the solvent and thus are available for various molecular interactions. Altering these amino acids could result in greater stability of the protein by eliminating or occluding protease-sensitive sites. Amino acid substitutions that change the surface charge of domain 1 could alter ion channel efficiency or alter interactions with the brush border membrane or with other portions of the toxin molecule, allowing binding or insertion to be more effective.

According to this invention, base substitutions are made in the underlying cry3Bb nucleic acid residues in order to change particular codons of the corresponding polypeptides, and particularly, in those loop regions between α -helices. The insecticidal activity of a crystal protein ultimately dictates the level of crystal protein required for effective insect control. The potency of an insecticidal protein should be maximized as much as possible in order to provide for its economic and efficient utilization in the field. The increased potency of an insecticidal protein in a bioinsecticide formulation would be expected to improve the field performance of the bioinsecticide product. Alternatively, increased

potency of an insecticidal protein in a bioinsecticide formulation may promote use of reduced amounts of bioinsecticide per unit area of treated crop, thereby allowing for more cost-effective use of the bioinsecticide product. When expressed in planta, the production of crystal proteins with improved insecticidal activity can be expected to improve plant resistance to susceptible insect pests.

4.2 Methods for Culturing *B. Thuringiensis* to Produce Crystal Proteins

The *B. thuringiensis* strains described herein may be cultured using standard known media and fermentation techniques. Upon completion of the fermentation cycle, the bacteria may be harvested by first separating the *B. thuringiensis* spores and crystals from the fermentation broth by means well known in the art. The recovered *B. thuringiensis* spores and crystals can be formulated into a wettable powder, a liquid concentrate, granules or other formulations by the addition of surfactants, dispersants, inert carriers and other components to facilitate handling and application for particular target pests. The formulation and application procedures are all well known in the art.

4.3 Recombinate Host Cells for Expression of Cry* Genes

The nucleotide sequences of the subject invention can be introduced into a wide variety of microbial hosts. Expression of the toxin gene results, directly or indirectly, in the intracellular production and maintenance of the pesticide. With suitable hosts, e.g., *Pseudomonas*, the microbes can be applied to the sites of coleopteran insects where they will proliferate and be ingested by the insects. The result is a control of the unwanted insects. Alternatively, the microbe hosting the toxin gene can be treated under conditions that prolong the activity of the toxin produced in the cell. The treated cell then can be applied to the environment of target pest(s). The resulting product retains the toxicity of the *B. thuringiensis* toxin.

Suitable host cells, where the pesticide-containing cells will be treated to prolong the activity of the toxin in the cell when the then treated cell is applied to the environment of target pest(s), may include either prokaryotes or eukaryotes, normally being limited to those cells which do not produce substances toxic to higher organisms, such as mammals. However, organisms which produce substances toxic to higher organisms could be used, where the toxin is unstable or the level of application sufficiently low as to avoid any possibility or toxicity to a mammalian host. As hosts, of particular interest will be the prokaryotes and the lower eukaryotes, such as fungi. Illustrative prokaryotes, both Gram-negative and Gram-positive, include Enterobacteriaceae, such as *Escherichia*, *Erwinia*, *Shigella*, *Salmonella*, and *Proteus*; Bacillaceae; Rhizobiaceae, such as *Rhizobium*; Spirillaceae, such as *Photobacterium*, *Zymomonas*, *Serratia*, *Aeromonas*, *Vibrio*, *Desulfovibrio*, *Spirillum*; Lactobacillaceae; Pseudomonadaceae, such as *Pseudomonas* and *Acetobacter*; Azotobacteraceae, Actinomycetales, and Nitrobacteraceae. Among eukaryotes are fungi, such as *Phycomycetes* and *Ascomycetes*, which includes yeast, such as *Saccharomyces* and *Schizosaccharomyces*; and Basidiomycetes yeast, such as *Rhodotorula*, *Aureobasidium*, *Sporobolomyces*, and the like.

Characteristics of particular interest in selecting a host cell for purposes of production include ease of introducing the *B. thuringiensis* gene into the host, availability of expression systems, efficiency of expression, stability of the

pesticide in the host, and the presence of auxiliary genetic capabilities. Characteristics of interest for use as a pesticide micro-capsule include protective qualities for the pesticide, such as thick cell walls, pigmentation, and intracellular packaging or formation of inclusion bodies; leaf affinity; lack of mammalian toxicity; attractiveness to pests for ingestion; ease of killing and fixing without damage to the toxin; and the like. Other considerations include ease of formulation and handling, economics, storage stability, and the like.

Host organisms of particular interest include yeast, such as *Rhodotorula* sp., *Aureobasidium* sp., *Saccharomyces* sp., and *Sporobolomyces* sp.; phylloplane organisms such as *Pseudomonas* sp., *Erwinia* sp. and *Flavobacterium* sp.; or such other organisms as *Escherichia*, *Lactobacillus* sp., *Bacillus* sp., *Streptomyces* sp., and the like. Specific organisms include *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Saccharomyces cerevisiae*, *B. thuringiensis*, *Escherichia coli*, *B. subtilis*, *B. megaterium*, *B. cereus*, *Streptomyces lividans* and the like.

Treatment of the microbial cell, e.g., a microbe containing the *B. thuringiensis* toxin gene, can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the toxin, nor diminish the cellular capability in protecting the toxin. Examples of chemical reagents are halogenating agents, particularly halogens of atomic no. 17–80. More particularly, iodine can be used under mild conditions and for sufficient time to achieve the desired results. Other suitable techniques include treatment with aldehydes, such as formaldehyde and glutaraldehyde; anti-infectives, such as zephiran chloride and cetylpyridinium chloride; alcohols, such as isopropyl and ethanol; various histologic fixatives, such as Lugol's iodine, Bouin's fixative, and Helly's fixatives, (see e.g., Humason, 1967); or a combination of physical (heat) and chemical agents that preserve and prolong the activity of the toxin produced in the cell when the cell is administered to the host animal. Examples of physical means are short wavelength radiation such as γ -radiation and X-radiation, freezing, UV irradiation, lyophilization, and the like. The cells employed will usually be intact and be substantially in the proliferative form when treated, rather than in a spore form, although in some instances spores may be employed.

Where the *B. thuringiensis* toxin gene is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, it is essential that certain host microbes be used. Microorganism hosts are selected which are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplane) of one or more crops of interest. These microorganisms are selected so as to be capable of successfully competing in the particular environment (crop and other insect habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably, provide for improved protection of the pesticide from environmental degradation and inactivation.

A large number of microorganisms are known to inhabit the phylloplane (the surface of the plant leaves) and/or the rhizosphere (the soil surrounding plant roots) of a wide variety of important crops. These microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, e.g., genera *Bacillus* (including the species and subspecies *B. thuringiensis* kurstaki HD-1, *B. thuringiensis* kurstaki HD-73, *B. thuringiensis* sotto, *B. thuringiensis* berliner, *B. thuringiensis* thuringiensis, *B. thuringiensis* tolworthi, *B. thuringiensis*

dendrolimus, *B. thuringiensis alesti*, *B. thuringiensis galleriae*, *B. thuringiensis aizawai*, *B. thuringiensis subtoxius*, *B. thuringiensis entomocidus*, *B. thuringiensis tenebrionis* and *B. thuringiensis san diego*; *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*, *Zanthomonas*, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylophilus*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*, *Arthrobacter*, *Azotobacter*, *Leuconostoc*, and *Alcaligenes*; fungi, particularly yeast, e.g., genera *Saccharomyces*, *Cryptococcus*, *Kluyveromyces*, *Sporobolomyces*, *Rhodotorula*, and *Aureobasidium*. Of particular interest are such phytosphere bacterial species as *Pseudomonas syringae*, *Pseudomonas fluorescens*, *Serratia marcescens*, *Acetobacter xylinum*, *Agrobacterium tumefaciens*, *Rhodobacter sphaeroides*, *Xanthomonas campestris*, *Rhizobium melioli*, *Alcaligenes eutrophus*, and *Azotobacter vinlandii*; and phytosphere yeast species such as *Rhodotorula rubra*, *R. glutinis*, *R. marina*, *R. aurantiaca*, *Cryptococcus albidus*, *C. diffluens*, *C. laurentii*, *Saccharomyces rosei*, *S. pretoriensis*, *S. cerevisiae*, *Sporobolomyces roseus*, *S. odoratus*, *Kluyveromyces veronae*, and *Aureobasidium pollulans*.

4.4 Definitions

In accordance with the present invention, nucleic acid sequences include and are not limited to DNA (including and not limited to genomic or extragenomic DNA), genes, RNA (including and not limited to mRNA and tRNA), nucleosides, and suitable nucleic acid segments either obtained from native sources, chemically synthesized, modified, or otherwise prepared by the hand of man. The following words and phrases have the meanings set forth below.

A, an: In accordance with long standing patent law convention, the words "a" and "an" when used in this application, including the claims, denotes "one or more".

Broad-spectrum: Refers to a wide range of insect species.

Broad-spectrum activity: The toxicity towards a wide range of insect species.

Expression: The combination of intracellular processes, including transcription and translation undergone by a coding DNA molecule such as a structural gene to produce a polypeptide.

Insecticidal activity: The toxicity towards insects.

Insecticidal specificity: The toxicity exhibited by a crystal protein or proteins, microbe or plant, towards multiple insect species.

Intraorder specificity: The toxicity of a particular crystal protein towards insect species within an Order of insects (e.g., Order Coleoptera).

Interorder specificity: The toxicity of a particular crystal protein towards insect species of different Orders (e.g., Orders Coleoptera and Diptera).

LC₅₀: The lethal concentration of crystal protein that causes 50% mortality of the insects treated.

LC₉₅: The lethal concentration of crystal protein that causes 95% mortality of the insects treated.

Promoter: A recognition site on a DNA sequence or group of DNA sequences that provide an expression control element for a structural gene and to which RNA polymerase specifically binds and initiates RNA synthesis (transcription) of that gene.

Regeneration: The process of growing a plant from a plant cell (e.g., plant protoplast or explant).

Structural gene: A gene that is expressed to produce a polypeptide.

Transformation: A process of introducing an exogenous DNA sequence (e.g., a vector, a recombinant DNA molecule) into a cell or protoplast in which that exogenous DNA is incorporated into a chromosome or is capable of autonomous replication.

Transformed cell: A cell whose DNA has been altered by the introduction of an exogenous DNA molecule into that cell.

Transgenic cell: Any cell derived or regenerated from a transformed cell or derived from a transgenic cell. Exemplary transgenic cells include plant calli derived from a transformed plant cell and particular cells such as leaf, root, stem, e.g., somatic cells, or reproductive (germ) cells obtained from a transgenic plant.

Transgenic plant: A plant or progeny thereof derived from a transformed plant cell or protoplast, wherein the plant DNA contains an introduced exogenous DNA molecule not originally present in a native, non-transgenic plant of the same strain. The terms "transgenic plant" and "transformed plant" have sometimes been used in the art as synonymous terms to define a plant whose DNA contains an exogenous DNA molecule. However, it is thought more scientifically correct to refer to a regenerated plant or callus obtained from a transformed plant cell or protoplast as being a transgenic plant, and that usage will be followed herein.

Vector: A DNA molecule capable of replication in a host cell and/or to which another DNA segment can be operatively linked so as to bring about replication of the attached segment. A plasmid is an exemplary vector.

As used herein, the designations "CryIII" and "Cry3" are synonymous, as are the designations "CryIIIB2" and "Cry3Bb." Likewise, the inventors have utilized the generic term Cry3Bb* to denote any and all Cry3Bb variants which comprise amino acid sequences modified in the protein. Similarly, cry3Bb* is meant to denote any and all nucleic acid segments and/or genes which encode a Cry3Bb* protein, etc.

4.5 Preparation of Cry3* Polynucleotides

Once the structure of the desired peptide to be mutagenized has been analyzed using one or more of the design strategies disclosed herein, it will be desirable to introduce one or more mutations into either the protein or, alternatively, into the DNA sequence encoding the protein for the purpose of producing a mutated protein with altered bioinsecticidal properties.

To that end, the present invention encompasses both site-specific mutagenesis methods and random mutagenesis of a nucleic acid segment encoding a crystal protein in the manner described herein. In particular, methods are disclosed for the mutagenesis of nucleic acid segments encoding the amino acid sequences using one or more of the design strategies described herein. Using the assay methods described herein, one may then identify mutants arising from these procedures which have improved insecticidal properties or altered specificity, either intraorder or inter-order.

The means for mutagenizing a DNA segment encoding a crystal protein are well-known to those of skill in the art. Modifications may be made by random, or site-specific mutagenesis procedures. The nucleic acid may be modified by altering its structure through the addition or deletion of one or more nucleotides from the sequence.

Mutagenesis may be performed in accordance with any of the techniques known in the art such as and not limited to

synthesizing an oligonucleotide having one or more mutations within the sequence of a particular crystal protein. A "suitable host" is any host which will express Cry3Bb, such as and not limited to *B. thuringiensis* and *E. coli*. Screening for insecticidal activity, in the case of Cry3Bb includes and is not limited to coleopteran-toxic activity which may be screened for by techniques known in the art.

In particular, site-specific mutagenesis is a technique useful in the preparation of individual peptides, or biologically functional equivalent proteins or peptides, through specific mutagenesis of the underlying DNA. The technique further provides a ready ability to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the DNA. Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Typically, a primer of about 17 to about 75 nucleotides or more in length is preferred, with about 10 to about 25 or more residues on both sides of the junction of the sequence being altered.

In general, the technique of site-specific mutagenesis is well known in the art, as exemplified by various publications. As will be appreciated, the technique typically employs a phage vector which exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phage are readily commercially available and their use is generally well known to those skilled in the art. Double stranded plasmids are also routinely employed in site directed mutagenesis which eliminates the step of transferring the gene of interest from a plasmid to a phage.

In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector or melting apart of two strands of a double stranded vector which includes within its sequence a DNA sequence which encodes the desired peptide. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically. This primer is then annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as *E. coli* polymerase 1 Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform or transfect appropriate cells, such as *E. coli* cells, and clones are selected which include recombinant vectors bearing the mutated sequence arrangement. A genetic selection scheme was devised by Kunkel et al (1987) to enrich for clones incorporating the mutagenic oligonucleotide. Alternatively, the use of PCRTM with commercially available thermostable enzymes such as Taq polymerase may be used to incorporate a mutagenic oligonucleotide primer into an amplified DNA fragment that can then be cloned into an appropriate cloning or expression vector. The PCRTM-mediated mutagenesis procedure of Tomic et al. (1990) and Upender et al. (1995) provide two examples of such protocols. A PCRTM employing a thermostable ligase in addition to a thermostable polymerase may also be used to incorporate a phosphorylated mutagenic oligonucleotide into an amplified DNA fragment that may then be cloned into an appropriate cloning or expression vector. The mutagenesis procedure described by Michael (1994) provides an example of one such protocol.

The preparation of sequence variants of the selected peptide-encoding DNA segments using site-directed mutagenesis is provided as a means of producing potentially useful species and is not meant to be limiting as there are other ways in which sequence variants of peptides and the DNA sequences encoding them may be obtained. For example, recombinant vectors encoding the desired peptide sequence may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants.

As used herein, the term "oligonucleotide directed mutagenesis procedure" refers to template-dependent processes and vector-mediated propagation which result in an increase in the concentration of a specific nucleic acid molecule relative to its initial concentration, or in an increase in the concentration of a detectable signal, such as amplification. As used herein, the term "oligonucleotide directed mutagenesis procedure" is intended to refer to a process that involves the template-dependent extension of a primer molecule. The term template dependent process refers to nucleic acid synthesis of an RNA or a DNA molecule wherein the sequence of the newly synthesized strand of nucleic acid is dictated by the well-known rules of complementary base pairing (see, for example, Watson, 1987). Typically, vector mediated methodologies involve the introduction of the nucleic acid fragment into a DNA or RNA vector, the clonal amplification of the vector, and the recovery of the amplified nucleic acid fragment. Examples of such methodologies are provided by U.S. Pat. No. 4,237, 224, specifically incorporated herein by reference in its entirety.

A number of template dependent processes are available to amplify the target sequences of interest present in a sample. One of the best known amplification methods is the polymerase chain reaction (PCRTM) which is described in detail in U.S. Pat. Nos. 4,683,195, 4,683,202 and 4,800,159 (each of which is specifically incorporated herein by reference in its entirety). Briefly, in PCRTM, two primer sequences are prepared which are complementary to regions on opposite complementary strands of the target sequence. An excess of deoxynucleoside triphosphates are added to a reaction mixture along with a DNA polymerase (e.g., Taq polymerase). If the target sequence is present in a sample, the primers will bind to the target and the polymerase will cause the primers to be extended along the target sequence by adding on nucleotides. By raising and lowering the temperature of the reaction mixture, the extended primers will dissociate from the target to form reaction products, excess primers will bind to the target and to the reaction products and the process is repeated. Preferably a reverse transcriptase PCRTM amplification procedure may be performed in order to quantify the amount of mRNA amplified. Polymerase chain reaction methodologies are well known in the art.

Another method for amplification is the ligase chain reaction (referred to as LCR), disclosed in Eur. Pat. Appl. Publ. No. 320,308, incorporated herein by reference in its entirety. In LCR, two complementary probe pairs are prepared, and in the presence of the target sequence, each pair will bind to opposite complementary strands of the target such that they abut. In the presence of a ligase, the two probe pairs will link to form a single unit. By temperature cycling, as in PCRTM, bound ligated units dissociate from the target and then serve as "target sequences" for ligation of excess probe pairs. U.S. Pat. No. 4,883,750, specifically incorporated herein by reference in its entirety, describes an alternative method of amplification similar to LCR for binding probe pairs to a target sequence.

Qbeta Replicase™, described in Intl. Pat. Appl. Publ. No. PCT/US87/00880, incorporated herein by reference in its entirety, may also be used as still another amplification method in the present invention. In this method, a replicative sequence of RNA which has a region complementary to that of a target is added to a sample in the presence of an RNA polymerase. The polymerase will copy the replicative sequence which can then be detected.

An isothermal amplification method, in which restriction endonucleases and ligases are used to achieve the amplification of target molecules that contain nucleotide 5'-[α-thio] triphosphates in one strand of a restriction site (Walker et al., 1992, incorporated herein by reference in its entirety), may also be useful in the amplification of nucleic acids in the present invention.

Strand Displacement Amplification (SDA) is another method of carrying out iso-thermal amplification of nucleic acids which involves multiple rounds of strand displacement and synthesis, i.e., nick translation. A similar method, called Repair Chain Reaction (RCR) is another method of amplification which may be useful in the present invention and involves annealing several probes throughout a region targeted for amplification, followed by a repair reaction in which only two of the four bases are present. The other two bases can be added as biotinylated derivatives for easy detection. A similar approach is used in SDA

Sequences can also be detected using a cyclic probe reaction (CPR). In CPR, a probe having 3' and 5' end sequences of non-Cry-specific DNA and an internal sequence of a Cry-specific RNA is hybridized to DNA which is present in a sample. Upon hybridization, the reaction is treated with RNaseH, and the products of the probe identified as distinctive products generating a signal which are released after digestion. The original template is annealed to another cycling probe and the reaction is repeated. Thus, CPR involves amplifying a signal generated by hybridization of a probe to a cry-specific expressed nucleic acid

Still other amplification methods described in Great Britain Pat. Appl. No. 2 202 328, and in Intl. Pat. Appl. Publ. No. PCT/US89/01025, each of which is incorporated herein by reference in its entirety, may be used in accordance with the present invention. In the former application, "modified" primers are used in a PCT™ like, template and enzyme dependent synthesis. The primers may be modified by labeling with a capture moiety (e.g., biotin) and/or a detector moiety (e.g., enzyme). In the latter application, an excess of labeled probes are added to a sample. In the presence of the target sequence, the probe binds and is cleaved catalytically. After cleavage, the target sequence is released intact to be bound by excess probe. Cleavage of the labeled probe signals the presence of the target sequence

Other nucleic acid amplification procedures include transcription-based amplification systems (TAS) (Kwoh et al., 1989; Intl. Pat. Appl. Publ. No. WO 88/10315, incorporated herein by reference in its entirety), including nucleic acid sequence based amplification (NASBA) and 3SR. In NASBA, the nucleic acids can be prepared for amplification by standard phenol/chloroform extraction, heat denaturation of a sample, treatment with lysis buffer and minispin columns for isolation of DNA and RNA or guanidinium chloride extraction of RNA. These amplification techniques involve annealing a primer which has crystal protein-specific sequences. Following polymerization, DNA/RNA hybrids are digested with RNase H while double stranded DNA molecules are heat denatured again. In either case the single stranded DNA is made fully double stranded by

addition of second crystal protein-specific primer, followed by polymerization. The double stranded DNA molecules are then multiply transcribed by a polymerase such as T7 or SP6. In an isothermal cyclic reaction, the RNAs are reverse transcribed into double stranded DNA, and transcribed once against with a polymerase such as T7 or SP6. The resulting products, whether truncated or complete, indicate crystal protein-specific sequences.

Eur. Pat. Appl. Publ. No. 329,822, incorporated herein by reference in its entirety, disclose a nucleic acid amplification process involving cyclically synthesizing single-stranded RNA ("ssRNA"), ssDNA, and double-stranded DNA (dsDNA), which may be used in accordance with the present invention. The ssRNA is a first template for a first primer oligonucleotide, which is elongated by reverse transcriptase (RNA-dependent DNA polymerase). The RNA is then removed from resulting DNA:RNA duplex by the action of ribonuclease H (RNase H, an RNase specific for RNA in a duplex with either DNA or RNA). The resultant ssDNA is a second template for a second primer, which also includes the sequences of an RNA polymerase promoter (exemplified by T7 RNA polymerase) 5' to its homology to its template. This primer is then extended by DNA polymerase (exemplified by the large "Klenow" fragment of E. coli DNA polymerase I), resulting as a double-stranded DNA ("dsDNA") molecule, having a sequence identical to that of the original RNA between the primers and having additionally, at one end, a promoter sequence. This promoter sequence can be used by the appropriate RNA polymerase to make many RNA copies of the DNA. These copies can then re-enter the cycle leading to very swift amplification. With proper choice of enzymes, this amplification can be done isothermally without addition of enzymes at each cycle. Because of the cyclical nature of this process, the starting sequence can be chosen to be in the form of either DNA or RNA

Intl. Pat. Appl. Publ. No. WO 89/06700, incorporated herein by reference in its entirety, disclose a nucleic acid sequence amplification scheme based on the hybridization of a promoter/primer sequence to a target single-stranded DNA ("ssDNA") followed by transcription of many RNA copies of the sequence. This scheme is not cyclic; i.e., new templates are not produced from the resultant RNA transcripts. Other amplification methods include "RACE" (Frohman, 1990), and "one-sided PCR™" (Ohara, 1989) which are well-known to those of skill in the art.

Methods based on ligation of two (or more) oligonucleotides in the presence of nucleic acid having the sequence of the resulting "di-oligonucleotide", thereby amplifying the di-oligonucleotide (Wu and Dean, 1996, incorporated herein by reference in its entirety), may also be used in the amplification of DNA sequences of the present invention.

4.6 Phage-resistant Variants

In certain embodiments, one may desired to prepare one or more phage resistant variants of the B. thuringiensis mutants prepared by the methods described herein. To do so, an aliquot of a phage lysate is spread onto nutrient agar and allowed to dry. An aliquot of the phage sensitive bacterial strain is then plated directly over the dried lysate and allowed to dry. The plates are incubated at 30° C. The plates are incubated for 2 days and, at that time, numerous colonies could be seen growing on the agar. Some of these colonies are picked and subcultured onto nutrient agar plates. These apparent resistant cultures are tested for resistance by cross streaking with the phage lysate. A line of the phage lysate is streaked on the plate and allowed to dry. The presumptive

resistant cultures are then streaked across the phage line. Resistant bacterial cultures show no lysis anywhere in the streak across the phage line after overnight incubation at 30° C. The resistance to phage is then reconfirmed by plating a lawn of the resistant culture onto a nutrient agar plate. The sensitive strain is also plated in the same manner to serve as the positive control. After drying, a drop of the phage lysate is plated in the center of the plate and allowed to dry. Resistant cultures showed no lysis in the area where the phage lysate has been placed after incubation at 30° C. for 24 hours.

4.7 Crystal Protein Compositions as Insecticides and Methods of Use

Order Coleoptera comprises numerous beetle species including ground beetles, reticulated beetles, skin and larder beetles, long-horned beetles, leaf beetles, weevils, bark beetles, ladybird beetles, soldier beetles, stag beetles, water scavenger beetles, and a host of other beetles. A brief taxonomy of the Order is given at the website <http://www.ncbi.nlm.nih.gov/Taxonomy/tax.html>.

Particularly important among the Coleoptera are the agricultural pests included within the infraorders Chrysomeliformia and Cucujiformia. Members of the infraorder Chrysomeliformia, including the leaf beetles (Chrysomelidae) and the weevils (Curculionidae), are particularly problematic to agriculture, and are responsible for a variety of insect damage to crops and plants. The infraorder Cucujiformia includes the families Coccinellidae, Cucujidae, Lagridae, Meloidae, Rhipiphoridae, and Tenebrionidae. Within this infraorder, members of the family Chrysomelidae (which includes the genera *Exema*, *Chrysomela*, *Oreina*, *Chrysolina*, *Leptinotarsa*, *Gonioctena*, *Oulema*, *Monozia*, *Ophraella*, *Cerotoma*, *Diabrotica*, and *Lachnaia*), are well-known for their potential to destroy agricultural crops.

As the toxins of the present invention have been shown to be effective in combatting a variety of members of the order Coleoptera, the inventors contemplate that the insects of many Coleopteran genera may be controlled or eradicated using the polypeptide compositions described herein. Likewise, the methods described herein for generating modified polypeptides having enhanced insect specificity may also be useful in extending the range of the insecticidal activity of the modified polypeptides to other insect species within, and outside of, the Order Coleoptera.

As such, the inventors contemplate that the crystal protein compositions disclosed herein will find particular utility as insecticides for topical and/or systemic application to field crops, including but not limited to rice, wheat, alfalfa, corn (maize), soybeans, tobacco, potato, barley, canola (rapeseed), sugarbeet, sugarcane, flax, rye, oats, cotton, sunflower; grasses, such as pasture and turf grasses; fruits, citrus, nuts, trees, shrubs and vegetables; as well as ornamental plants, cacti, succulents, and the like.

Disclosed and claimed is a composition comprising an insecticidally-effective amount of a Cry3Bb* crystal protein composition. The composition preferably comprises the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54,

SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, or SEQ ID NO:108, or biologically-functional equivalents thereof.

The insecticide composition may also comprise a Cry3Bb* crystal protein that is encoded by a nucleic acid sequence having the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, or SEQ ID NO:108, or, alternatively, a nucleic acid sequence which hybridizes to the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, or SEQ ID NO:107 under conditions of moderate stringency.

The insecticidal compositions may comprise one or more *B. thuringiensis* cell types, or one or more cultures of such cells, or alternatively, a mixture of one or more *B. thuringiensis* cells which express one or more of the novel crystal proteins of the invention in combination with another insecticidal composition. In certain aspects it may be desirable to prepare compositions which contain a plurality of crystal proteins, either native or modified, for treatment of one or more types of susceptible insects. The *B. thuringiensis* cells of the invention can be treated prior to formulation to prolong the insecticidal activity when the cells are applied to the environment of the target insect(s). Such treatment can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the insecticide, nor diminish the cellular capability in protecting the insecticide. Examples of chemical reagents are halogenating agents, particularly halogens of atomic no. 17-80. More particularly, iodine can be used under mild conditions and for sufficient time to achieve the desired results. Other suitable techniques include treatment with aldehydes, such as formaldehyde and glutaraldehyde; anti-infectives, such as zephiran chloride; alcohols, such as isopropyl and ethanol; various histologic fixatives, such as Bouin's fixative and Helly's fixative (see Humason, 1967); or a combination of physical (heat) and chemical agents that prolong the activity of the δ -endotoxin produced in the cell when the cell is applied to the environment of the target pest(s). Examples of physical means are short wavelength radiation such as gamma-radiation and X-radiation, freezing, UV irradiation, lyophilization, and the like.

The inventors contemplate that any formulation methods known to those of skill in the art may be employed using the proteins disclosed herein to prepare such bioinsecticide compositions. It may be desirable to formulate whole cell preparations, cell extracts, cell suspensions, cell homogenates, cell lysates, cell supernatants, cell filtrates, or

cell pellets of a cell culture (preferably a bacterial cell culture such as a *B. thuringiensis* cell culture described in Table 3) that expresses one more cry3Bb* DNA segments to produce the encoded Cry3Bb* protein(s) or peptide(s). The methods for preparing such formulations are known to those of skill in the art, and may include, e.g., desiccation, lyophilization, homogenization, extraction, filtration, centrifugation, sedimentation, or concentration of one or more cultures of bacterial cells, such as *B. thuringiensis* cells described in Table 3, which express the Cry3Bb* peptide(s) of interest.

In one preferred embodiment, the bioinsecticide composition comprises an oil flowable suspension comprising lysed or unlysed bacterial cells, spores, or crystals which contain one or more of the novel crystal proteins disclosed herein. Preferably the cells are *B. thuringiensis* cells, however, any such bacterial host cell expressing the novel nucleic acid segments disclosed herein and producing a crystal protein is contemplated to be useful, such as *Bacillus* spp., including *B. megaterium*, *B. subtilis*; *B. cereus*, *Escherichia* spp., including *E. coli*, and/or *Pseudomonas* spp., including *P. cepacia*, *P. aeruginosa*, and *P. fluorescens*. Alternatively, the oil flowable suspension may consist of a combination of one or more of the following compositions: lysed or unlysed bacterial cells, spores, crystals, and/or purified crystal proteins.

In a second preferred embodiment, the bioinsecticide composition comprises a water dispersible granule or powder. This granule or powder may comprise lysed or unlysed bacterial cells, spores, or crystals which contain one or more of the novel crystal proteins disclosed herein. Preferred sources for these compositions include bacterial cells such as *B. thuringiensis* cells, however, bacteria of the genera *Bacillus*, *Escherichia*, and *Pseudomonas* which have been transformed with a DNA segment disclosed herein and expressing the crystal protein are also contemplated to be useful. Alternatively, the granule or powder may consist of a combination of one or more of the following compositions: lysed or unlysed bacterial cells, spores, crystals, and/or purified crystal proteins.

In a third important embodiment, the bioinsecticide composition comprises a wettable powder, spray, emulsion, colloid, aqueous or organic solution, dust, pellet, or colloidal concentrate. Such a composition may contain either unlysed or lysed bacterial cells, spores, crystals, or cell extracts as described above, which contain one or more of the novel crystal proteins disclosed herein. Preferred bacterial cells are *B. thuringiensis* cells, however, bacteria such as *B. megaterium*, *B. subtilis*, *B. cereus*, *B. coli*, or *Pseudomonas* spp. cells transformed with a DNA segment disclosed herein and expressing the crystal protein are also contemplated to be useful. Such dry forms of the insecticidal compositions may be formulated to dissolve immediately upon wetting, or alternatively, dissolve in a controlled-release, sustained-release, or other time-dependent manner. Alternatively, such a composition may consist of a combination of one or more of the following compositions: lysed or unlysed bacterial cells, spores, crystals, and/or purified crystal proteins.

In a fourth important embodiment, the bioinsecticide composition comprises an aqueous solution or suspension or cell culture of lysed or unlysed bacterial cells, spores, crystals, or a mixture of lysed or unlysed bacterial cells, spores, and/or crystals, such as those described above which contain one or more of the novel crystal proteins disclosed herein. Such aqueous solutions or suspensions may be provided as a concentrated stock solution which is diluted prior to application, or alternatively, as a diluted solution ready-to-apply.

For these methods involving application of bacterial cells, the cellular host containing the Crystal protein gene(s) may be grown in any convenient nutrient medium, where the DNA construct provides a selective advantage, providing for a selective medium so that substantially all or all of the cells retain the *B. thuringiensis* gene. These cells may then be harvested in accordance with conventional ways. Alternatively, the cells may be treated prior to harvesting.

When the insecticidal compositions comprise *B. thuringiensis* cells, spores, and/or crystals containing the modified crystal protein(s) of interest, such compositions may be formulated in a variety of ways. They may be employed as wettable powders, granules or dusts, by mixing with various inert materials, such as inorganic minerals (phyllsilicates, carbonates, sulfates, phosphates, and the like) or botanical materials (powdered comcobs, rice hulls, walnut shells, and the like). The formulations may include spreader-sticker adjuvants, stabilizing agents, other pesticidal additives, or surfactants. Liquid formulations may be aqueous-based or non-aqueous and employed as foams, suspensions, emulsifiable concentrates, or the like. The ingredients may include rheological agents, surfactants, emulsifiers, dispersants, or polymers.

Alternatively, the novel Cry3Bb-derived mutated crystal proteins may be prepared by native or recombinant bacterial expression systems in vitro and isolated for subsequent field application. Such protein may be either in crude cell lysates, suspensions, colloids, etc., or alternatively may be purified, refined, buffered, and/or further processed, before formulating in an active biocidal formulation. Likewise, under certain circumstances, it may be desirable to isolate crystals and/or spores from bacterial cultures expressing the crystal protein and apply solutions, suspensions, or colloidal preparations of such crystals and/or spores as the active bioinsecticidal composition.

Another important aspect of the invention is a method of controlling coleopteran insects which are susceptible to the novel compositions disclosed herein. Such a method generally comprises contacting the insect or insect population, colony, etc., with an insecticidally-effective amount of a Cry3Bb* crystal protein composition. The method may utilize Cry3Bb* crystal proteins such as those disclosed in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, or SEQ ID NO:108, or biologically functional equivalents thereof.

Alternatively, the method may utilize one or more Cry3Bb* crystal proteins which are encoded by the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, SEQ ID NO:101, or SEQ ID

NO:107, or by one or more nucleic acid sequences which hybridize to the subsequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, SEQ ID NO:101, or SEQ ID NO:107, under conditions of moderate, or higher, stringency. The methods for identifying sequences which hybridize to those disclosed under conditions of moderate or higher stringency are well-known to those of skill in the art, and are discussed herein.

Regardless of the method of application, the amount of the active component(s) are applied at an insecticidally-effective amount, which will vary depending on such factors as, for example, the specific coleopteran insects to be controlled, the specific plant or crop to be treated, the environmental conditions, and the method, rate, and quantity of application of the insecticidally-active composition.

The insecticide compositions described may be made by formulating either the bacterial cell, crystal and/or spore suspension, or isolated protein component with the desired agriculturally-acceptable carrier. The compositions may be formulated prior to administration in an appropriate means such as lyophilized, freeze-dried, desiccated, or in an aqueous carrier, medium or suitable diluents, such as saline or other buffer. The formulated compositions may be in the form of a dust or granular material, or a suspension in oil (vegetable or mineral), or water or oil/water emulsions, or as a wettable powder, or in combination with any other carrier material suitable for agricultural application. Suitable agricultural carriers can be solid or liquid and are well known in the art. The term "agriculturally-acceptable carrier" covers all adjuvants, e.g., inert components, dispersants, surfactants, tackifiers, binders, etc. that are ordinarily used in insecticide formulation technology; these are well known to those skilled in the insecticide formulation. The formulations may be mixed with one or more solid or liquid adjuvants and prepared by various means, e.g. by homogeneously mixing, blending and/or grinding the insecticidal composition with suitable adjuvants using conventional formulation techniques.

The insecticidal compositions of this invention are applied to the environment of the target coleopteran insect, typically onto the foliage of the plant or crop to be protected, by conventional methods, preferably by spraying. The strength and duration of insecticidal application will be set with regard to conditions specific to the particular pest(s), crop(s) to be treated with particular environmental conditions. The proportional ratio of active ingredient to carrier will naturally depend on the chemical nature, solubility, and stability of the insecticidal composition, as well as the particular formulation contemplated.

Other application techniques, e.g., dusting, sprinkling, soaking, soil injection, soil tilling, seed coating, seedling coating, spraying, aerating, misting, atomizing, and the like, are also feasible and may be required under certain circumstances such as e.g., insects that cause root or stalk infestation, or for application to delicate vegetation or ornamental plants. These application procedures are also well-known to those of skill in the art.

The insecticidal composition of the invention may be employed in the method of the invention singly or in

combination with other compounds, including and not limited to other pesticides. The method of the invention may also be used in conjunction with other treatments such as surfactants, detergents, polymers or time-release formulations. The insecticidal compositions of the present invention may be formulated for either systemic or topical use.

The concentration of insecticidal composition which is used for environmental, systemic, or foliar application will vary widely depending upon the nature of the particular formulation, means of application, environmental conditions, and degree of biocidal activity. Typically, the bioinsecticidal composition will be present in the applied formulation at a concentration of at least about 1% by weight and may be up to and including about 99% by weight. Dry formulations of the compositions may be from about 1% to about 99% or more by weight of the composition, while liquid formulations may generally comprise from about 1% to about 99% or more of the active ingredient by weight. Formulations which comprise intact bacterial cells will generally contain from about 10^4 to about 10^2 cells/mg.

The insecticidal formulation may be administered to a particular plant or target area in one or more applications as needed, with a typical field application rate per hectare ranging on the order of from about 1 g to about 1 kg, 2 kg, 5, kg, or more of active ingredient.

4.8 Nucleic Acid Segments as Hybridization Probes and Primers

In addition to their use in directing the expression of crystal proteins or peptides of the present invention, the nucleic acid sequences contemplated herein also have a variety of other uses. For example, they also have utility as probes or primers in nucleic acid hybridization embodiments. As such, it is contemplated that nucleic acid segments that comprise a sequence region that consists of at least a 14 nucleotide long contiguous sequence that has the same sequence as, or is complementary to, a 14 nucleotide long contiguous DNA segment of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, SEQ ID NO:101, or SEQ ID NO:107 will find particular utility. Longer contiguous identical or complementary sequences, e.g., those of about 20, 30, 40, 50, 100, 200, 500, 1000, 2000, 5000, 10000 etc. (including all intermediate lengths and up to and including full-length sequences will also be of use in certain embodiments.

The ability of such nucleic acid probes to specifically hybridize to crystal protein-encoding sequences will enable them to be of use in detecting the presence of complementary sequences in a given sample. However, other uses are envisioned, including the use of the sequence information for the preparation of mutant species primers, or primers for use in preparing other genetic constructions.

Nucleic acid molecules having sequence regions consisting of contiguous nucleotide stretches of 10-14, 15-20, 30, 50, or even of 100-200 nucleotides or so, identical or complementary to DNA sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ

ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, SEQ ID NO:101, or SEQ ID NO:107 are particularly contemplated as hybridization probes for use in e.g., Southern and Northern blotting. Smaller fragments will generally find use in hybridization embodiments, wherein the length of the contiguous complementary region may be varied, such as between about 10–14 and about 100 or 200 nucleotides, but larger contiguous complementary stretches may be used, according to the length complementary sequences one wishes to detect.

The use of a hybridization probe of about 14 nucleotides in length allows the formulation of a duplex molecule that is both stable and selective. Molecules having contiguous complementary sequences over stretches greater than 14 bases in length are generally preferred, though, in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 15 to 20 contiguous nucleotides, or even longer where desired.

Of course, fragments may also be obtained by other techniques such as, e.g., by mechanical shearing or by restriction enzyme digestion. Small nucleic acid segments or fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, as is commonly practiced using an automated oligonucleotide synthesizer. Also, fragments may be obtained by application of nucleic acid reproduction technology, such as the PCR™ technology of U.S. Pat. No. 4,683,195 and 4,683,202 (each incorporated herein by reference), by introducing selected sequences into recombinant vectors for recombinant production, and by other recombinant DNA techniques generally known to those of skill in the art of molecular biology.

Accordingly, the nucleotide sequences of the invention may be used for their ability to selectively form duplex molecules with complementary stretches of DNA fragments. Depending on the application envisioned, one will desire to employ varying conditions of hybridization to achieve varying degrees of selectivity of probe toward target sequence. For applications requiring high selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids, e.g., one will select relatively low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.15 M NaCl at temperatures of about 50° C. to about 70° C. Such selective conditions tolerate little, if any, mismatch between the probe and the template or target strand, and would be particularly suitable for isolating crystal protein-encoding DNA segments. Detection of DNA segments via hybridization is well-known to those of skill in the art, and the teachings of U.S. Pat. Nos. 4,965,188 and 5,176,995 (each incorporated herein by reference) are exemplary of the methods of hybridization analyses. Teachings such as those found in the texts of Maloy et al., 1994; Segal 1976; Prokop, 1991; and Kuby, 1994, are particularly relevant.

Of course, for some applications, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template or where one seeks to isolate crystal protein-encoding sequences from related

species, functional equivalents, or the like, less stringent hybridization conditions will typically be needed in order to allow formation of the heteroduplex. In these circumstances, one may desire to employ conditions such as about 0.15 M to about 0.9 M salt, at temperatures ranging from about 20° C. to about 55° C. Cross-hybridizing species can thereby be readily identified as positively hybridizing signals with respect to control hybridizations. In any case, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

In certain embodiments, it will be advantageous to employ nucleic acid sequences of the present invention in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including fluorescent, radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of giving a detectable signal. In preferred embodiments, one will likely desire to employ a fluorescent label or an enzyme tag, such as urease, alkaline phosphatase or peroxidase, instead of radioactive or other environmental undesirable reagents. In the case of enzyme tags, calorimetric indicator substrates are known that can be employed to provide a means visible to the human eye or spectrophotometrically, to identify specific hybridization with complementary nucleic acid-containing samples.

In general, it is envisioned that the hybridization probes described herein will be useful both as reagents in solution hybridization as well as in embodiments employing a solid phase. In embodiments involving a solid phase, the test DNA (or RNA) is adsorbed or otherwise affixed to a selected matrix or surface. This fixed, single-stranded nucleic acid is then subjected to specific hybridization with selected probes under desired conditions. The selected conditions will depend on the particular circumstances based on the particular criteria required (depending, for example, on the G+C content, type of target nucleic acid, source of nucleic acid, size of hybridization probe, etc.) Following washing of the hybridized surface so as to remove nonspecifically bound probe molecules, specific hybridization is detected, or even quantitated, by means of the label.

4.9 Characteristics of Modified Cry3 δ -endotoxins

The present invention provides novel polypeptides that define a whole or a portion of a *B. thuringiensis* cry3Bb.60, cry3Bb.11221, cry3Bb.11222, cry3Bb.11223, cry3Bb.11224, cry3Bb.11225, cry3Bb.11226, cry3Bb.11227, cry3Bb.11228, cry3Bb.11229, cry3Bb.11230, cry3Bb.11231, cry3Bb.11232, cry3Bb.11233, cry3Bb.11234, cry3Bb.11235, cry3Bb.11236, cry3Bb.11237, cry3Bb.11238, cry3Bb.11239, cry3Bb.11241, cry3Bb.11242, cry3Bb.11032, cry3Bb.11035, cry3Bb.11036, cry3Bb.11046, cry3Bb.11048, cry3Bb.11051, cry3Bb.11057, cry3Bb.11058, cry3Bb.11081, cry3Bb.11082, cry3Bb.11083, cry3Bb.11084, cry3Bb.11095, and cry3Bb.11098-encoded crystal protein.

4.10 Crystal Protein Nomenclature

The inventors arbitrarily assigned the designations Cry3Bb.60, Cry3Bb.11221, Cry3Bb.11222, Cry3Bb.11223, Cry3Bb.11224, Cry3Bb.11225, Cry3Bb.11226, Cry3Bb.11227, Cry3Bb.11228, Cry3Bb.11229,

Cry3Bb.11230, Cry3Bb.11231, Cry3Bb.11232, Cry3Bb.11233, Cry3Bb.11234, Cry3Bb.11235, Cry3Bb.11236, Cry3Bb.11237, Cry3Bb.11238, Cry3Bb.11239, Cry3Bb.11241, Cry3Bb.11242, Cry3Bb.11032, Cry3Bb.11035, Cry3Bb.11036, Cry3Bb.11046, Cry3Bb.11048, Cry3Bb.11051, Cry3Bb.11057, Cry3Bb.11058, Cry3Bb.11081, Cry3Bb.11082, Cry3Bb.11083, Cry3Bb.11084, Cry3Bb.11095, and Cry3Bb.11098 to the novel proteins of the invention.

Likewise, the arbitrary designations of Cry3Bb.60 cry3Bb.11221, cry3Bb.11222, cry3Bb.11223, cry3Bb.11224, cry3Bb.11225, cry3Bb.11226, cry3Bb.11227, cry3Bb.11228, cry3Bb.11229, cry3Bb.11230, cry3Bb.11231, cry3Bb.11232, cry3Bb.11233, cry3Bb.11234, cry3Bb.11235, cry3Bb.11236, cry3Bb.11237, cry3Bb.11238, cry3Bb.11239, cry3Bb.11241, cry3Bb.11242, cry3Bb.11032, cry3Bb.11035, cry3Bb.11036, cry3Bb.11046, cry3Bb.11048, cry3Bb.11051, cry3Bb.11057, cry3Bb.11058, cry3Bb.11081, cry3Bb.11082, cry3Bb.11083, cry3Bb.11084, cry3Bb.11095 and Cry3Bb.11098 have been assigned to the novel nucleic acid sequences which encode these polypeptides, respectively. While format assignment of gene and protein designations based on the revised nomenclature of crystal protein endotoxins (Table 1) may be made by the committee on the nomenclature of *B. thuringiensis*, any re-designations of the compositions of the present invention are also contemplated to be fully within the scope of the present disclosure.

4.11 Transformed Host Cells and Transgenic Plants

A bacterium, a yeast cell, or a plant cell or a plant transformed with an expression vector of the present invention is also contemplated. A transgenic bacterium, yeast cell, plant cell or plant derived from such a transformed or transgenic cell is also one aspect of the invention.

Such transformed host cells are often desirable for use in the production of endotoxins and for expression of the various DNA gene constructs disclosed herein. In some aspects of the invention, it is often desirable to modulate, regulate, or otherwise control the expression of the gene segments disclosed herein. Such methods are routine to those of skill in the molecular genetic arts. Typically, when increased or over-expression of a particular gene is desired, various manipulations may be employed for enhancing the expression of the messenger RNA, particularly by using an active promoter, as well as by employing sequences, which enhance the stability of the messenger RNA in the particular transformed host cell.

Typically, the initiation and translational termination region will involve stop codon(s), a terminator region, and optionally, a polyadenylation signal. In the direction of transcription, namely in the 5' to 3' direction of the coding or sense sequence, the construct will involve the transcriptional regulatory region, if any, and the promoter, where the regulatory region may be either 5' or 3' of the promoter, the ribosomal binding site, the initiation codon, the structural gene having an open reading frame in phase with the initiation codon, the stop codon(s), the polyadenylation signal sequence, if any, and the terminator region. This sequence as a double strand may be used by itself for transformation of a microorganism host, but will usually be included with a DNA sequence involving a marker, where the second DNA sequence may be joined to the δ -endotoxin expression construct during introduction of the DNA into the host.

By a marker is intended a structural gene which provides for selection of those hosts which have been modified or transformed. The marker will normally provide for selective advantage, for example, providing for biocide resistance, e.g., resistance to antibiotics or heavy metals; complementation, so as to provide prototrophy to an auxotrophic host, or the like. Preferably, complementation is employed, so that the modified host may not only be selected, but may also be competitive in the field. One or more markers may be employed in the development of the constructs, as well as for modifying the host. The organisms may be further modified by providing for a competitive advantage against other wild-type microorganisms in the field. For example, genes expressing metal chelating agents, e.g., siderophores, may be introduced into the host along with the structural gene expressing the δ -endotoxin. In this manner, the enhanced expression of a siderophore may provide for a competitive advantage for the δ -endotoxin-producing host, so that it may effectively compete with the wild-type microorganisms and stably occupy a niche in the environment.

Where no functional replication system is present, the construct will also include a sequence of at least 50 basepairs (bp), preferably at least about 100 bp, and usually not more than about 1000 bp of a sequence homologous with a sequence in the host. In this way, the probability of legitimate recombination is enhanced, so that the gene will be integrated into the host and stably maintained by the host. Desirably, the δ -endotoxin gene will be in close proximity to the gene providing for complementation as well as the gene providing for the competitive advantage. Therefore, in the event that a δ -endotoxin gene is lost, the resulting organism will be likely to also lose the complementing gene and/or the gene providing for the competitive advantage, so that it will be unable to compete in the environment with the gene retaining the intact construct.

The crystal protein-encoding gene can be introduced between the transcriptional and translational initiation region and the transcriptional and translational termination region, so as to be under the regulatory control of the initiation region. This construct will be included in a plasmid, which will include at least one replication system, but may include more than one, where one replication system is employed for cloning during the development of the plasmid and the second replication system is necessary for functioning in the ultimate host. In addition, one or more markers may be present, which have been described previously. Where integration is desired, the plasmid will desirably include a sequence homologous with the host genome.

The transformants can be isolated in accordance with conventional ways, usually employing a selection technique, which allows for selection of the desired organism as against unmodified organisms or transferring organisms, when present. The transformants then can be tested for pesticidal activity.

Suitable host cells, where the pesticide-containing cells will be treated to prolong the activity of the δ -endotoxin in the cell when the then treated cell is applied to the environment of the target pest(s), may include either prokaryotes or eukaryotes, normally being limited to those cells which do not produce substances toxic to higher organisms, such as mammals. However, organisms which produce substances toxic to higher organisms could be used, where the δ -endotoxin is unstable or the level of application sufficiently low as to avoid any possibility of toxicity to a mammalian host. As hosts, of particular interest will be the prokaryotes and the lower eukaryotes, such as fungi, ill-

trative prokaryotes, both Gram-negative and -positive, include Enterobacteriaceae, such as *Escherichia*, *Erwinia*, *Shigella*, *Salmonella*, and *Proteus*; Bacillaceae; Rhizobiales, such as *Rhizobium*; Spirillaceae, such as *Photobacterium*, *Zymomonas*, *Serratia*, *Aeromonas*, *Vibrio*, *Desulfovibrio*, *Spirillum*; Lactobacillaceae; phylloplane organisms such as members of the Pseudomonadaceae (including *Pseudomonas* spp. and *Acetobacter* spp.); Azotobacteraceae and Nitrobacteraceae, *Flavobacterium* spp.; members of the Bacillaceae such as *Lactobacillus* spp., *Bifidobacterium*, and *Bacillus* spp., and the like. Particularly preferred host cells include *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *Escherichia coli*, *Bacillus subtilis*, and the like.

Among eukaryotes are fungi, such as *Phycomycetes* and *Ascomycetes*, which includes yeast, such as *Schizosaccharomyces*; and *Basidiomycetes*, *Rhodotorula*, *Aureobasidium*, *Sporobolomyces*, *Saccharomyces* spp., and *Sporobolomyces* spp.

Characteristics of particular interest in selecting a host cell for purposes of production include ease of introducing the δ -endotoxin gene into the host, availability of expression systems, efficiency of expression, stability of the pesticide in the host, and the presence of auxiliary genetic capabilities. Characteristics of interest for use as a pesticide microcapsule include protective qualities for the pesticide, such as thick cell walls, pigmentation, and intracellular packaging or formation of inclusion bodies; leaf affinity; lack of mammalian toxicity; attractiveness to pests for ingestion; ease of killing and fixing without damage to the δ -endotoxin; and the like. Other considerations include ease of formulation and handling, economics, storage stability, and the like.

The cell will usually be intact and be substantially in the proliferative form when treated, rather than in a spore form, although in some instance spores may be employed. Treatment of the recombinant microbial cell can be done as disclosed infra. The treated cells generally will have enhanced structural stability which will enhance resistance to environmental conditions.

Genes or other nucleic acid segments, as disclosed herein, can be inserted into host cells using a variety of techniques which are well known in the art. For example, a large number of cloning vectors comprising a replication system in *E. coli* and a marker that permits selection of the transformed cells are available for preparation for the insertion of foreign genes into higher organisms, including plants. The vectors comprise, for example, pBR322, pUC series, M13mp series, pACY184, etc. Accordingly, the sequence coding for the δ -endotoxin can be inserted into the vector at a suitable restriction site. The resulting plasmid is used for transformation into *E. coli*. The *E. coli* cells are cultivated in a suitable nutrient medium, then harvested and lysed. The plasmid is recovered. Sequence analysis, restriction analysis, electrophoresis, and other biochemical-molecular biological methods are generally carried out as methods of analysis. After each manipulation, the DNA sequence used can be cleaved and joined to the next DNA sequence. Each plasmid sequence can be cloned in the same or other plasmids. Depending on the method of inserting desired genes into the plant, other DNA sequences may be necessary.

Methods for DNA transformation of plant cells include *Agrobacterium*-mediated plant transformation, protoplast transformation, gene transfer into pollen, injection into reproductive organs, injection into immature embryos and particle bombardment. Each of these methods has distinct

advantages and disadvantages. Thus, one particular method of introducing genes into a particular plant strain may not necessarily be the most effective for another plant strain, but it is well known which methods are useful for a particular plant strain.

Suitable methods are believed to include virtually any method by which DNA can be introduced into a cell, such as by *Agrobacterium* infection, direct delivery of DNA such as, for example, by PEG-mediated transformation of protoplasts (Omiruelleh et al., 1993), by desiccation/inhibition-mediated DNA uptake, by electroporation, by agitation with silicon carbide fibers, by acceleration of DNA coated particles, etc. In certain embodiments, acceleration methods are preferred and include, for example, microprojectile bombardment and the like.

Technology for introduction of DNA into cells is well-known to those of skill in the art. Four general methods for delivering a gene into cells have been described: (1) chemical methods (Graham and van der Eb, 1973; Zatlouka et al., 1992); (2) physical methods such as microinjection (Capecchi, 1980), electroporation (Wong and Neumann, 1982; Fromm et al., 1985) and the gene gun (Johnston and Tang, 1994; Fynan et al., 1993); (3) viral vectors (Clapp, 1993; Lu et al., 1993; Eglitis and Anderson, 1988; Eglitis et al., 1988); and (4) receptor-mediated mechanisms (Curiel et al., 1991; 1992; Wagner et al., 1992).

A large number of techniques are available for inserting DNA into a plant host cell. Those techniques include transformation with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as transformation agent, fusion, injection, or electroporation as well as other possible methods. If *agrobacteria* are used for the transformation, the DNA to be inserted has to be cloned into special plasmids, namely either into an intermediate vector or into a binary vector. The intermediate vectors can be integrated into the Ti or Ri plasmid by homologous recombination owing to sequences that are homologous to sequences in the T-DNA. The Ti or Ri plasmid also comprises the vir region necessary for the transfer of the T-DNA.

Intermediate vectors cannot replicate themselves in *agrobacteria*. The intermediate vector can be transferred into *Agrobacterium tumefaciens* by means of a helper plasmid (conjugation). Binary vectors can replicate themselves both in *E. coli* and in *agrobacteria*. They comprise a selection marker gene and a linker or polylinker which are framed by the right and left T-DNA border regions. They can be transformed directly into *agrobacteria* (Holsters et al., 1978). The *agrobacterium* used as host cell is to comprise a plasmid carrying a vir region. The vir region is necessary for the transfer of the T-DNA into the plant cell. Additional T-DNA may be contained. The bacterium so transformed is used for the transformation of plant cells. Plant explants can advantageously be cultivated with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* for the transfer of the DNA into the plant cell. Whole plants can then be regenerated from the infected plant material (for example, pieces of leaf, segments of stalk, roots, but also protoplasts or suspension-cultivated cells) in a suitable medium, which may contain antibiotics or biocides for selection. The plants so obtained can then be tested for the presence of the inserted DNA. No special demands are made of the plasmids in the case of injection and electroporation. It is possible to use ordinary plasmids, such as, for example, pUC derivatives. If, for example, the Ti or Ri plasmid is used for the transformation of the plant cell, then at least the right border, but often the right and the left border of the Ti or Ri plasmid T-DNA, has to be joined as the flanking region of the genes to be inserted.

The use of T-DNA for the transformation of plant cells has been intensively researched and sufficiently described in Eur. Pat. Appl. No. EP 120 516; Hockema (1985); An et al., 1985, Herrera-Estrella et al., (1983), Bevan et al., (1983), and Klee et al., (1985).

A particularly useful Ti plasmid cassette vector for transformation of dicotyledonous plants consists of the enhanced CaMV35S promoter (EN35S) and the 3' end including polyadenylation signals from a soybean gene encoding the α' -subunit of β -conglycinin. Between these two elements is a multilinker containing multiple restriction sites for the insertion of genes of interest.

The vector preferably contains a segment of pBR322 which provides an origin of replication in *E. coli* and a region for homologous recombination with the disarmed T-DNA in *Agrobacterium* strain ACO; the oriV region from the broad host range plasmid RK1; the streptomycin/spectinomycin resistance gene from Tn7; and a chimeric NPTII gene, containing the CaMV35S promoter and the nopaline synthase (NOS) 3' end, which provides kinamycin resistance in transformed plant cells.

Optionally, the enhanced CaMV35S promoter may be replaced with the 1.5 kb manopine synthase (MA) promoter (Velten et al., 1984). After incorporation of a DNA construct into the vector, it is introduced into *A. tumefaciens* strain ACO which contains a disarmed Ti plasmid. Cointegrate Ti plasmid vectors are selected and subsequently may be used to transform a dicotyledonous plant.

A. tumefaciens ACO is a disarmed strain similar to pTiB6SE described by Fraley et al. (1985). For construction of aCO the starting *Agrobacterium* strain was the strain A208 which contains a nopaline-type Ti plasmid. The Ti plasmid was disarmed in a manner similar to that described by Fraley et al. (1985) so that essentially all of the native T-DNA was removed except for the leaf border and a few hundred base pairs of T-DNA inside the left border. The remainder of the T-DNA extending to a point just beyond the right border was replaced with a novel piece of DNA including (from left to right) a segment of pBR322, the oriV region from plasmid RK2, and the kanamycin resistance gene from Tn601. The pBR322 and oriV segments are similar to those segments and provide a region of homology for cointegrated formation.

Once the inserted DNA has been integrated in the genome, it is relatively stable there and, as a rule, does not come out again. It normally contains a selection marker that confers on the transformed plant cells resistance to a biocide or an antibiotic, such as kinamycin, G 418, bleomycin, hygromycin, or chloramphenicol, inter alia. The individually employed marker should accordingly permit the selection of transformed cells rather than cells that do not contain the inserted DNA.

4.11.1 Electroporation

The application of brief, high-voltage electric pulses to a variety of animal and plant cells leads to the formation of nanometer-sized pores in the plasma membrane. DNA is taken directly into the cell cytoplasm either through these pores or as a consequence of the redistribution of membrane components that accompanies closure of the pores. Electroporation can be extremely efficient and can be used both for transient expression of clones genes and for establishment of cell lines that carry integrated copies of the gene of interest. Electroporation, in contrast to calcium phosphate-mediated transfection and protoplast fusion, frequently gives rise to cell lines that carry one, or at most a few, integrated copies of the foreign DNA.

The introduction of DNA by means of electroporation, is well-known to those of skill in the art. In this method, certain cell wall-degrading enzymes, such as pectin-degrading enzymes, are employed to render the target recipient cells more susceptible to transformation by electroporation than untreated cells. Alternatively, recipient cells are made more susceptible to transformation, by mechanical wounding. To effect transformation by electroporation one may employ either friable tissues such as a suspension culture of cells, or embryogenic callus, or alternatively, one may transform immature embryos or other organized tissues directly. One would partially degrade the cell walls of the chosen cells by exposing them to pectin-degrading enzymes (pectolyases) or mechanical wounding in a controlled manner. Such cells would then be recipient to DNA transfer by electroporation, which may be carried out at this stage, and transformed cells then identified by a suitable selection or screening protocol dependent on the nature of the newly incorporated DNA.

4.11.2 Microprojectile Bombardment

A further advantageous method for delivering transforming DNA segments to plant cells is microprojectile bombardment. In this method, particles may be coated with nucleic acids and delivered into cells by a propelling force. Exemplary particles include those comprised of tungsten, gold, platinum, and the like.

An advantage of microprojectile bombardment, in addition to it being an effective means of reproducibly stably transforming monocots, is that neither the isolation of protoplasts (Cristou et al., 1988) nor the susceptibility to *Agrobacterium* infection is required. An illustrative embodiment of a method for delivering DNA into maize cells by acceleration is a Biolistics Particle Delivery System, which can be used to propel particles coated with DNA or cells through a screen, such as a stainless steel or Nytex screen, onto a filter surface covered with corn cells cultured in suspension. The screen disperses the particles so that they are not delivered to the recipient cells in large aggregates. It is believed that a screen intervening between the projectile apparatus and the cells to be bombarded reduces the size of projectiles aggregate and may contribute to a higher frequency of transformation by reducing damage inflicted on the recipient cells by projectiles that are too large.

For the bombardment, cells in suspension are preferably concentrated on filters or solid culture medium. Alternatively, immature embryos or other target cells may be arranged on solid culture medium. The cells to be bombarded are positioned at an application distance below the macroprojectile stopping plate. If desired, one or more screens are also positioned between the acceleration device and the cells to be bombarded. Through the use of techniques set forth herein one may obtain up to 1000 or more foci of cells transiently expressing a marker gene. The number of cells in a focus which express the exogenous gene product 48 hours post-bombardment often range from 1 to 10 and average 1 to 3.

In bombardment transformation, one may optimize the prebombardment culturing conditions and the bombardment parameters to yield the maximum numbers of stable transformants. Both the physical and biological parameters for bombardment are important in this technology. Physical factors are those that involve manipulating the DNA/microprojectile precipitate or those that affect the flight and velocity of either the macro or microprojectiles. Biological factors include all steps involved in manipulation of cells before and immediately after bombardment, the osmotic

adjustment of target cells to help alleviate the trauma associated with bombardment, and also the nature of the transforming DNA, such as linearized DNA or intact supercoiled plasmids. It is believed that the pre-bombardment manipulations are especially important for successful transformation of immature embryos.

Accordingly, it is contemplated that one may wish to adjust various of the bombardment parameters in small scale studies to fully optimize the conditions. One may particularly wish to adjust physical parameters such as gap distance, flight distance, tissue distance, and helium pressure. One may also minimize the trauma reduction factors (TRFs) by modifying conditions which influence the physiological state of the recipient cells and which may therefore influence transformation and integration efficiencies. For example, the osmotic state, tissue hydration and the subculture stage or cell cycle of the recipient cells may be adjusted for optimum transformation. The execution of other routine adjustments will be known to those of skill in the art in light of the present disclosure.

4.11.3 Agrobacterium-mediated Transfer

Agrobacterium-mediated transfer is a widely applicable system for introducing genes into plant cells because the DNA can be introduced into whole plant tissue, thereby bypassing the need for regeneration of an intact plant from a protoplast. The use of Agrobacterium-mediated plant integrating vectors to introduce DNA into plant cells is well known in the art. See, for example, the methods described (Fraley et al., 1985; Rogers et al., 1987). Further, the integration of *th* Ti-DNA is a relatively precise process resulting in few rearrangements. The region of DNA to be transferred is defined by the border sequences, and intervening DNA is usually inserted into the plant genome as described (Spielmann et al., 1986; Jorgensen et al., 1987).

Modern Agrobacterium transformation vectors are capable of replication *E. coli* as well as Agrobacterium, allowing for convenient manipulations as described (Klee et al., 1985). Moreover, recent technological advances in vectors for Agrobacterium-mediated gene transfer have improved the arrangement of genes and restriction sites in the vectors to facilitate construction of vectors capable of expressing various polypeptide coding genes. The vectors described (Rogers et al., 1987), have convenient multi-linker regions flanked by a promoter and a polyadenylation site for direct expression of inserted polypeptide coding genes and are suitable for present purposes. In addition, Agrobacterium containing both armed and disarmed Ti genes can be used for the transformations. In those plant strains where Agrobacterium-mediated transformation is efficient, it is the method of choice because of the facile and defined nature of the gene transfer.

Agrobacterium-mediate transformation of leaf disks and other tissues such as cotyledons and hypocotyls appears to be limited to plants that Agrobacterium naturally infects. Agrobacterium-mediated transformation to most efficient in dicotyledonous plants. Few monocots appear to be natural hosts for Agrobacterium, although transgenic plants have been produced in asparagus using Agrobacterium vectors as described (Bytebier et al., 1987). Therefore, commercially important cereal grains such as rice, corn, and wheat must usually be transformed using alternative methods. However, as mentioned above, the transformation of asparagus using Agrobacterium can also be achieved (see, for example, Bytebier et al., 1987).

A transgenic plant formed using Agrobacterium transformation methods typically contains a single gene on one

chromosome. Such transgenic plants can be referred to as being heterozygous for the added gene. However, inasmuch as use of the word "heterozygous" usually implies the presence of a complementary gene at the same locus of the second chromosome of a pair of chromosomes, and there is no such gene in a plant containing one added gene, as here, it is believed that a more accurate name for such a plant is an independent segregant, because the added, exogenous gene segregates independently during mitosis and meiosis.

More preferred is a transgenic plant that is homozygous for the added structural gene; i.e., a transgenic plant that contains two added genes, one gene at the same locus on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by sexually mating (selfing) an independent segregant transgenic plant that contains a single added gene, germinating some of the seed produced and analyzing the resulting plants produced for enhanced carboxylase activity relative to a control (native, non-transgenic) or an independent segregant transgenic plant.

It is to be understood that two different transgenic plants can also be mated to produce offspring that contain two independently segregating added, exogenous genes. Selfing of appropriate progeny can produce plants that are homozygous for both added, exogenous genes that encode a polypeptide of interest. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated.

Transformation of plant protoplasts can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation, and combination of these treatments (see, e.g., Potrykus et al., 1985; Lorz et al., 1985; Fromm et al., 1985; Uchimiya et al., 1986; Callis et al., 1987; Marcotte et al., 1988).

Application of these systems to different plant strains depends upon the ability to regenerate that particular plant strain from protoplasts. Illustrative methods for the regeneration of cereals from protoplasts are described (Fijimura et al., 1985; Toriyama et al., 1986; Yamada et al., 1986; Abdullab et al., 1986).

To transform plant strains that cannot be successfully regenerated from protoplasts, other ways to introduce DNA into intact cells or tissues can be utilized. For example, regeneration of cereals from immature embryos or explants can be effected as described (Vasil, 1988). In addition, "particle gun" or high-velocity microprojectile technology can be utilized (Vasil, 1992).

Using that latter technology, DAN is carried through the cell wall and into the cytoplasm on the surface of small metal particles as described (Klein et al., 1987; Klein et al., 1988; McCabe et al., 1988). The metal particles penetrate through several layers of cells and this allow the transformation of cells within tissue explants.

4.11.4 Gene Expression in Plants

Although great progress has been made in recent years with respect to preparation of transgenic plants which express bacterial proteins such as *B. thuringiensis* crystal proteins, the results of expressing native bacterial genes in plants are often disappointing. Unlike microbial genetics, little was known by early plant geneticists about the factors which afforded heterologous expression of foreign genes in plants. In recent years, however, several potential factors have been implicated as responsible in varying degrees for the level of protein expression from a particular coding sequence. For example, scientists now know that maintaining a significant level of a particular mRNA in the cell is

indeed a critical factor. Unfortunately, the causes for low steady state levels of mRNA encoding foreign proteins are many. First, full length RNA synthesis may not occur at a high frequency. This could, for example, be caused by the premature termination of RNA during transcription or due to unexpected mRNA processing during transcription. Second, full length RNA may be produced in the plant cell, but then processed (splicing, polyA addition) in the nucleus in a fashion that creates a nonfunctional mRNA. If the RNA is not properly synthesized, terminated and polyadenylated, it cannot move to the cytoplasm for translation. Similarly, in the cytoplasm, if mRNAs have reduced half lives (which are determined by their primary or secondary sequence) insufficient protein product will be produced. In addition, there is an effect, whose magnitude is uncertain, or translational efficiency on mRNA half-life. In addition, every RNA molecule folds into a particular structure, or perhaps family of structures, which is determined by its sequence. The particular structure of any RNA might lead to greater or lesser stability in the cytoplasm. Structure per se is probably also a determinant of mRNA processing in the nucleus. Unfortunately, it is impossible to predict, and nearly impossible to determine, the structure of any RNA (except for tRNA) in vitro or in vivo. However, it is likely that dramatically changing the sequence of an RNA will have a large effect on its folded structure. It is likely that structure per se or particular structural features also have a role in determining RNA stability.

To overcome these limitations in foreign gene expression, researchers have identified particular sequences and signals in RNAs that have the potential for having a specific effect on RNA stability. In certain embodiments of the invention, therefore, there is a desire to optimize expression of the disclosed nucleic acid segments in plants. One particular method of doing so, is by alternation of the bacterial gene to remove sequences or motifs which decrease expression in a transformed plant cell. The process of engineering a coding sequence for optimal expression in plants is often referred to as "plantizing" a DNA sequence.

Particularly problematic sequences are those which are A+T rich. Unfortunately, since *B. thuringiensis* has an A+T rich genome, native crystal protein gene sequences must often be modified for optimal expression in a plant. The sequence motif ATTA (or AUUUA as it appears in RNA) has been implicated as a destabilizing sequence in mammalian cell mRNA (Shaw and Kamen, 1986). Many short lived mRNAs have A+T rich 3' untranslated regions, and these regions often have the ATTA sequence, sometimes present in multiple copies as multimedia (e.g., ATTTATTTA . . .). Shaw and Kamen showed that the transfer of the 3' end of an unstable mRNA to a stable RNA (globin or VA1) decreased the stable RNA's half life dramatically. They further showed that a pentamer of ATTTA had a profound destabilizing effect on a stable message, and that this signal could exert its effect whether it was located at the 3' end or within the coding sequence. However, the number of ATTTA sequences and/or the sequence context in which they occur also appear to be important in determining whether they function as destabilizing sequences. Shaw and Kamen showed that a trimer of ATTTA had much less effect than a pentamer on mRNA stability and a dimer or a monomer had no effect on stability (Shaw and Kamen, 1987). Note that multimers of ATTA such as a pentamer automatically create an A+T rich region. This was shown to be a cytoplasmic effect, not nuclear. In other unstable mRNAs, the ATTTA sequence may be present in only a single copy, but it is often contained in an A+T rich region. From the animal cell data

collected to the date, it appears that ATTTA at least in some contexts is important in stability, but it is not yet possible to predict with occurrences of ATTTA are destabilizing elements or whether any of these effects are likely to be seen in plants.

Some studies on mRNA degradation in animal cells also indicate that RNA degradation may begin in some cases with nucleolytic attack in A+T rich regions. It is not clear if these cleavages occur at ATTTA sequences. There are also examples of mRNAs that have differential stability depending on the cell type in which they are expressed or on the stage within the cell cycle at which they are expressed. For example, histone mRNAs are stable during DNA synthesis but unstable if DNA synthesis is disrupted. The 3' end of some histidine mRNAs seems to be responsible for this effect (Pandey and Marzluff, 1987). It does not appear to be mediated by ATTTA, nor it is clear what controls the differential stability of this mRNA. Another example is the differential stability of IgG mRNA in B lymphocytes during B cell maturation (Genovese and Milcarek, 1998). A final example is the instability of a mutant β -thalassemic globin mRNA. In bone marrow cells, where this gene is normally expressed, the mutant mRNA is unstable, while the wild-type mRNA is stable. When the mutant gene is expressed in HeLa or L cells in vitro, the mutant mRNA shows no instability (Lim et al., 1988). These examples all provide evidence that mRNA stability can be mediated by cell type or cell cycle specific factors. Furthermore this type of instability is not yet associated with specific sequences. Given these uncertainties, it is not possible to predict which RNAs are likely to be unstable in a given cell. In addition, even the ATTTA motif may act differentially depending on the nature of the cell in which the RNA is present. Shaw and Kamen (1987) have reported that activation of protein kinase C can block degradation mediated by ATTTA.

The addition of a polyadenylation string to the 3' end is common to most eukaryotic mRNAs, both plant and animal. The currently accepted view of polyA addition is that the nascent transcript extends beyond the mature 3' terminus. Contained within this transcript are signals for polyadenylation and proper 3' end formation. This processing at the 3' end involves cleavage of the mRNA and addition of the polyA to the mature 3' end. By searching for consensus sequences near the polyA tract in both plant and animal mRNAs, it has been possible to identify consensus sequences that apparently are involved in polyA addition and 3' end cleavage. The same consensus sequences seem to be important to both of these processes. These signals are typically a variation on the sequence AATAAA. In animal cells, some variants of this sequence that are functional have been identified; in plant cells there seems to be an extended range of functional sequences (Wickens and Stephenson, 1984; Dean et al., 1986). Because all of these consensus sequences are variations on AATAAA, they all are A+T rich sequences. This sequence is typically found 15 to 20 bp before the polyA tract in a mature mRNA. Studies in animal cells indicate that this sequence is involved in both polyA addition and 3' maturation. Site directed mutations in this sequence can disrupt these functions (Conway and Wickens, 1988; Wickens et al., 1987). However, it has also been observed that sequences up to 50 to 100 bp 3' to the putative polyA signal are also required, i.e., a gene that has a normal AATAAA but has been replaced or disrupted downstream does not gel properly polyadenylated (Gil and Proudfoot, 1984; Sadofsky and Alwine, 1984; McDevitt et al., 1984). That is, the polyA signal itself is not sufficient for complete and proper processing. It is not yet known what specific

downstream sequences are required in addition to the polyA signal, or if there is a specific sequence that has time function. Therefore, sequence analysis can only identify potential polyA signals.

In naturally occurring mRNAs that are normally polyadenylated, it has been observed that disruption of this process, either by altering the polyA signal or other sequences in the mRNA, profound effects can be obtained in the level of functional mRNA. This has been observed in several naturally occurring mRNAs, with results that are gene-specific so far.

It has been shown that in natural mRNAs proper polyadenylation is important in mRNA accumulation, and that disruption of this process can effect mRNA levels significantly. However, insufficient knowledge exists to predict the effect of changes in a normal gene. In a heterologous gene, it is even harder to predict the consequences. However, it is possible that the putative sites identified are dysfunctional. That is, these sites may not act as proper polyA sites, but instead function as aberrant sites that give rise to unstable mRNAs.

In animal cell systems, AATAAA is by far the most communication signal identified in mRNAs upstream of the polyA, but at least four variants have also been found (Wickens and Stephenson, 1984). In plants, not nearly so much analysis has been done, but it is clear that multiple sequences similar to AATAAA can be used. The plant sites in Table 5 called major or minor refer only to the study of Dean et al. (1986) which analyzed only three types of plant gene. The designation of polyadenylation sites as major or minor refers only to the frequency of their occurrence as functional sites in naturally occurring genes that have been analyzed. In the case of plants this is a very limited database. It is hard to predict with any certainty that a site designated major or minor is more or less likely to function partially or completely when found in a heterologous gene such as those encoding the crystal proteins of the present invention.

TABLE 5

POLYADENYLATION SITES IN PLANT GENES		
PA	AATAAA	Major consensus site
P1A	AATAAT	Major plant site
P2A	AACCAA	Minor plant site
P3A	ATATAA	"
P4A	AATCAA	"
P5A	ATACTA	"
P6A	ATAAAA	"
P7A	ATGAAA	"
P8A	AAGCAT	"
P9A	ATTAAT	"
P10A	ATACAT	"
P11A	AAAATA	"
P12A	ATTAAA	Minor animal site
P13A	AATTAA	"
P14A	AATACA	"
P15A	CATAAA	"

The present invention provides a method for preparing synthetic plant genes which genes express their protein product at levels significantly higher than the wild-type gene which were commonly employed in plant transformation heretofore. In another aspect, the present invention also provides novel synthetic plant genes which encode non-polar proteins.

As described above, the expression of native *B. thuringiensis* genes in plants is often problematic. The nature of the coding sequences of *B. thuringiensis* genes distinguishes them from plant genes as well as many other heterologous

genes expressed in plants. In particular, *B. thuringiensis* genes are very rich (~62%) in adenine (A) and thymine (T) while plant genes and most other bacterial genes which have been expressed in plants are on the order of 45–55% A+T.

Due to the degeneracy of the genetic code and the limited number of codon choices for any amino acid, most of the "excess" A+T of the structural coding sequences of some *Bacillus* species are found in the third position of the codons. That is, gene of some *Bacillus* species have A or T as the third nucleotide in many codons. Thus A+T content in part can determine codon usage bias. In addition, it is clear that genes evolve for maximum function in the organism in which they evolve. This means that particular nucleotide sequences found in a gene from one organism, where they may play no role except to code for a particular stretch of amino acids, have the potential to be recognized as gene control elements in another organism (such as transcriptional promoters or terminators, polyA addition sites, intron splice sites, or specific mRNA degradation signals). It is perhaps surprising that such misread signals are not a more common feature of heterologous gene expression, but this can be explained in part by the relatively homogeneous A+T content (~50%) of many organisms. This A+T content plus the nature of the genetic code put clear constraints on the likelihood of occurrence of any particular oligonucleotide sequence. Thus, a gene from *E. coli* with a 50% A+T content is much less likely to contain any particular A+T rich segment than a gene from *B. thuringiensis*.

Typically, to obtain high-level expression of the S-endotoxin genes in plants, existing structural coding sequences ("structural gene") which codes for the S-endotoxin are modified by removal of ATTTA sequences and putative polyadenylation signals by site directed mutagenesis of the DNA comprising the structural gene. It is most preferred that substantially all the polyadenylation signals and ATTTA sequences are removed although enhanced expression levels are observed with only partial removal of either of the above identified sequences. Alternatively if a synthetic gene is prepared which codes for the expression of the subject protein, codons are selected to avoid the ATTTA sequence and putative polyadenylation signals. For purposes of the present invention putative polyadenylation signals include, but are not necessarily limited to, AATAAA, AATAAT, AACCAA, ATATAA, AATCAA, ATACTA, ATAAAA, ATGAAA, AAGCAT, ATTAAT, ATACAT, AAAATA, ATTAAA, AATTAA, AATACA and CATAAA. In replacing the ATTTA sequences and polyadenylation signals, codons are preferably utilized which avoid the codons which are rarely found in plant genomes.

The selected DNA sequence is scanned to identify regions with greater than four consecutive adenine (A) or thymine (T) nucleotides. The A+T regions are scanned for potential plant polyadenylation signals. Although the absence of five or more consecutive A or T nucleotides eliminates most plant polyadenylation signals, if there are more than one of the minor polyadenylation signals identified within ten nucleotides of each other, then the nucleotide sequence of this region is preferably altered to remove these signals while maintaining the original encoded amino acid sequence.

The second step is to consider the about 15 to about 30 or so nucleotide residues surrounding the A+T rich region identified in step one. If the A+T content of the surrounding region is less than 80%, the region should be examined for polyadenylation signals. Alteration of the region based on polyadenylation signals is dependent upon (1) the number of

polyadenylation signal present and (2) presence of a major plant polyadenylation signal.

The extended region is examined for the presence of plant polyadenylation signals. The polyadenylation signals are removed by site-directed mutagenesis of the DNA sequence. The extended region is also examined for multiple copies of the ATTTA sequence which are also removed by mutagenesis.

It is also preferred that regions comprising many consecutive A+T bases or G+C bases are disrupted since these regions are predicted to have a higher likelihood to form hairpin structure due to self-complementarity. Therefore, insertion of heterogeneous base pairs would reduce the likelihood of self-complementary secondary structure formation which are known to inhibit transcription and/or translation in some organisms. In most cases, the adverse effects may be minimized by using sequences which do not contain more than five consecutive A+T or G+C.

4.11.5 Synthetic Oligonucleotides for Mutagenesis

When oligonucleotides are used in the mutagenesis, it is desirable to maintain the proper amino acid sequence and reading frame, without introducing common restriction sites such as GgIII, HindIII, SacI, KpnI, EcoRI, NcoI, PstI and Sall into the modified gene. These restriction sites are found in poly-linker insertion sites of many cloning vectors. Of course, the introduction of new polyadenylation signals, ATTTA sequences or consecutive stretches of more than five A+T or G+C, should also be avoided. The preferred size for the oligonucleotides is about 40 to about 50 bases, but fragments ranging from about 18 to about 100 bases have been utilized. In most cases, a minimum of about 5 to about 8 base pairs of homology to the template DNA on both ends of the synthesized fragment are maintained to insure proper hybridization of the primer to the template. The oligonucleotides should avoid sequences longer than five base pairs A+T or G+C. Codons used in the replacement of wild-type codons should preferably avoid the TA or CG doublet wherever possible. Codons are selected from a plant preferred codon table (such as Table 6 below) so as to avoid codons which are rarely found in plant genomes, and efforts should be made to select codons to preferably adjust the G+C content to about 50%.

TABLE 6

PREFERRED CODON USAGE IN PLANTS		
Amino Acid	Codon	Percent Usage in Plants
ARG	CGA	7
	CGC	11
	CGG	5
	CGU	25
	AGA	29
LEU	AGG	23
	CUA	8
	CUC	20
	CUG	10
	CUU	28
SER	UUA	5
	UUG	30
	UCA	14
	UCC	26
	UCG	3
	UCU	21
	AGC	21
	AGU	15

TABLE 6-continued

PREFERRED CODON USAGE IN PLANTS		
Amino Acid	Codon	Percent Usage in Plants
THR	ACA	21
	ACC	41
	ACG	7
	ACU	31
PRO	CCA	45
	CCC	18
	CCG	9
	CCU	26
ALA	GCA	23
	GCC	32
	GCG	3
	GCU	41
GLY	GGA	32
	GGC	20
	GGG	11
	GGU	37
ILE	AUA	12
	AUC	45
	AUU	43
	GUA	9
VAL	GUC	20
	GUG	28
	GUU	43
	AAA	36
LYS	AAG	64
	AAC	72
ASN	AAU	28
	CAA	64
GLN	CAG	36
	CAC	65
HIS	CAU	35
	GAA	48
GLU	GAG	52
	GAC	48
ASP	GAU	52
	UAC	68
TYR	UAU	32
	UGC	78
CYS	UGU	22
	UUC	56
PHE	UUU	44
	AUG	100
MET	UGG	100
TRP		

Regions with many consecutive A+T bases or G+C bases are predicted to have a higher likelihood to form hairpin structures due to self-complementary. Disruption of these regions by the insertion of heterogeneous base pairs is preferred and should reduce the likelihood of the formation of self-complementary secondary structures such as hairpins which are known in some organisms to inhibit transcription (transcriptional terminators) and translation (attenuators).

Alternatively, a completely synthetic gene for a given amino acid sequence can be prepared, with regions of five or more consecutive A+T or G+C nucleotides being avoided. Codons are selected avoiding the TA and CG doublets in codons whenever possible. Codon usage can be normalized against a plant preferred codon usage table (such as Table 6) and the G+C content preferably adjusted to about 50%. The resulting sequence should be examined to ensure that there are minimal putative plant polyadenylation signals and ATTTA sequences. Restriction sites found in commonly used cloning vectors are also preferably avoided. However, placement of several unique restriction sites throughout the gene is useful for analysis of gene expression or construction of gene variants.

4.11.6 "Plantized" Gene Constructs

The expression of a plant gene which exists in double-stranded DNA form involves transcription of messenger

RNA (mRNA) from one strand of the DNA by RNA polymerase enzyme, and the subsequent processing of the mRNA primary transcript inside the nucleus. This processing involves a 3' non-translated region which adds polyadenylate nucleotides to the 3' end of the RNA. Transcription of DNA into mRNA is regulated by a region of DNA usually referred to as the "promoter." The promoter region contains a sequence of bases that signals RNA polymerase to associate with the DNA and to initiate the transcription of mRNA using one of the DNA strands as a template to make a corresponding strand of RNA.

A number of promoters which are active in plant cells have been described in the literature. These include the nopaline synthase (NOS) and octopine synthase (OCS) promoters (which are carried on tumor-inducing plasmids of *Agrobacterium tumefaciens*), the Cauliflower Mosaic Virus (CaMV) 19S and 35S promoters, the light-inducible promoter from the small subunit of ribulose bis-phosphate carboxylase (ssRUBISCO, a very abundant plant polypeptide) and the mannopine synthase (MAS) promoter (Velten et al., 1984 and Velten and Schnell, 1985). All of these promoters have been used to create various types of DNA constructs which have been expressed in plants (see e.g., Int. Pat. Appl. Publ. No. WO 84/02913).

Promoters which are known or are found to cause transcription of RNA in plant cells can be used in the present invention. Such promoters may be obtained from plants or plant viruses and include, but are not limited to, the CaMV35S promoter and promoters isolated from plant genes such as ssRUBISCO genes. As described below, it is preferred that the particular promoter selected should be capable of causing sufficient expression to result in the production of an effective amount of protein.

The promoters used in the DNA constructs (i.e. chimeric plant genes) of the present invention may be modified, if desired, to affect their control characteristics. For example, the CaMV35S promoter may be ligated to the portion of the ssRUBISCO gene that represents the expression of ssRUBISCO in the absence of light, to create a promoter which is active in leaves but not in roots. The resulting chimeric promoter may be used as described herein. For purposes of this description, the phrase "CaMV35S" promoter thus includes variations of CaMV35S promoter, e.g., promoters derived by means of ligation with operator regions, random or controlled mutagenesis, etc. Furthermore, the promoters may be altered to contain multiple "enhancer sequences" to assist in elevating gene expression.

The RNA produced by a DNA construct of the present invention also contains a 5' non-translated leader sequence. This sequence can be derived from the promoter selected to express the gene, and can be specifically modified so as to increase translation of the mRNA. The 5' non-translated regions can also be obtained from viral RNA's, from suitable eukaryotic genes, or from a synthetic gene sequence. The present invention is not limited to constructs, as presented in the following examples. Rather, the non-translated leader sequence can be part of the 5' end of the non-translated region of the coding sequence for the virus coat protein, or part of the promoter sequence, or can be derived from an unrelated promoter or coding sequence. In any case, it is preferred that the sequence flanking the initial site conform to the translational consensus sequence rules for enhanced translation initiation reported by Kozak (1984).

The cry DNA constructs of the present invention may also contain one or more modified or fully-synthetic structural

condign sequences which have been changed to enhance the performance of the cry gene in plants. The structural genes of the present invention may optionally encode a fusion protein comprising an amino-terminal chloroplast transit peptide or secretory signal sequence.

The DNA construct also contains a 3' non-translated region. The 3' non-translated region contains a polyadenylation signal which functions in plants to cause the addition of polyadenylate nucleotides to the 3' end of the viral RNA. Example of suitable 3' regions are (1) the 3' transcribed, non-translated regions containing the polyadenylation signal of *Agrobacterium* tumor-inducing (Ti) plasmid genes, such as the nopaline synthase (NOS) gene, and (2) plant genes like the soybean storage protein (7S) genes and the small subunit of the RuBP carboxylase (E9) gene.

4.12. Methods for Producing Insect-resistant Transgenic Plants

By transforming a suitable host cell, such as a plant cell, with a recombinant cry* gene-containing segment, the expression of the encoded crystal protein (i.e., a bacterial crystal protein or polypeptide having insecticidal activity against coleopterans) can result in the formation of insect-resistant plants.

By way of example, one may utilize an expression vector containing a coding region for a *B. thuringiensis* crystal protein and an appropriate selectable marker to transform a suspension of embryonic plant cells, such as wheat or corn cells using a method such as particle bombardment (Maddock et al., 1991; Vasil et al., 1992) to deliver the DNA coated on microprojectiles into the recipient cells. Transgenic plants are then regenerated from transformed embryonic calli that expresses the insecticidal proteins.

The formation of transgenic plants may also be accomplished using other methods of cell transformation which are known in the art such as *Agrobacterium*-mediated DNA transfer (Fraley et al., 1983). Alternatively, DNA can be introduced into plants by direct DNA transfer into pollen (Zhou et al., 1983; Hess, 1987; Luo et al., 1998), by injection of the DNA into reproductive organs of a plant (Pena et al., 1987), or by direct injection of DNA into the cells of immature embryos followed by the rehydration of desiccated embryos (Neuhaus et al., 1987; Benbrook et al. 1986).

The regeneration, development, and cultivation of plants from single plant protoplast transformants or from various transformed explants is well known in the art (Weissbach and Weissbach, 1988). This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The development or regeneration of plants containing the foreign, exogenous gene that encodes a polypeptide of interest introduced by *Agrobacterium* from leaf explants can be achieved by methods well known in the art such as described (Horsch et al., 1985). In this procedure, transformants are cultured in the presence of a selection agent and in a medium that induces the regeneration of shoots in the plant string being transformed as described (Fraley et al., 1983).

This procedure typically produces shoots within two to four months and those shoots are then transferred to an appropriate root-inducing medium containing the selective agent and an antibiotic to prevent bacterial growth. Shoots

that rooted in the presence of the selective agent to form plantlets are then transplanted to soil or other media to allow the production of roots. These procedures vary depending upon the particular plant strain employed, such variations being well known in the art.

Preferably, the regenerated plant are self-pollinated to provide homozygous transgenic plants, as discussed before. Otherwise, pollen obtained from the regenerated plant is crossed to seed-grown plants of agronomically important, preferably inbred lines. Conversely, pollen from plants of those important lines is used to pollinate regenerated plants. A transgenic plant of the present invention containing a desired polypeptide is cultivated using methods well known to one skilled in the art.

Such plants can form germ cells and transmit the transformed trait(s) to progeny plants. Likewise, transgenic plants can be grown in the normal manner and crossed with plants that have the same transformed hereditary factors or other hereditary factors. The resulting hybrid individuals have the corresponding phenotypic properties. A transgenic plant of this invention thus has an increased amount of a coding region (e.g., a mutated cry gene) that encodes the mutated Cry polypeptide of interest. A preferred transgenic plant is an independent segregant and can transmit that gene and its activity to its progeny. A more preferred transgenic plant is homozygous for that gene, and transmits that gene to all of its offspring on sexual mating.

Seed from a transgenic plant may be grown in the field or greenhouse, and resulting sexually mature transgenic plants are self-pollinated to generate true breeding plants. The progeny from these plants become true breeding lines that are evaluated for, by way of example, increased insecticidal capacity against coleopteran insects, preferably in the field, under a range of environment conditions. The inventors contemplate that the present invention will find particular utility in the creation of transgenic plants of commercial interest including various grasses, grains, fibers, tubers, legumens, ornamental plants, cacti, succulents, fruits, berries, and vegetables, as well as a number of nut- and fruit-bearing trees and plants.

4.13 Methods for Producing Combinatorial Cry3* Variants

Crystal protein mutants containing substitutions in one or more domains may be constructed via a number of techniques. For instance, sequences of highly related genes can be readily shuffled using the PCRTM-based technique described by Stemmer (1994). Alternatively, if suitable restriction sites are available, the mutations of one cry gene may be combined with the mutations of a second cry gene by routine subcloning methodologies. If a suitable restriction site is not available, one may be generated by oligonucleotide directed mutagenesis using any number of procedures known to those skilled in the art. Alternatively, splice-overlap extension PCRTM (Horton et al., 1989) may be used to combine mutations in different regions of a crystal protein. In this process, overlapping DNA fragments generated by the PCRTM and containing different mutations within their unique sequences may be annealed and used as a template for amplification using flanking primers to generate a hybrid gene sequence. Finally, cry* mutants may be combined by simply using one cry mutant as a template for oligonucleotide-directed mutagenesis using any number of protocols such as those described herein.

4.14 Isolating Homologous Gene and Gene Fragments

The genes and δ -endotoxins according to the subject invention include not only the full length sequences dis-

closed herein but also fragments of these sequences, or fusion proteins, which retain the characteristics insecticidal activity of the sequences specifically exemplified herein.

It should be apparent to a person skilled in the art that insecticidal δ -endotoxins can be identified and obtained through several means. The specific genes, or portions thereof, may be obtained from a culture depository, or constructed synthetically, for example, by use of a gene machine. Variations of these genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as Bal31 or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which code for active fragments may be obtained using a variety of other restriction enzymes. Proteases may be used to directly obtain active fragments of these δ -endotoxins.

Equivalent δ -endotoxins and/or genes encoding these equivalent δ -endotoxins can also be isolated from *Bacillus* strains and/or DNA libraries using the teachings provided herein. For example, antibodies to the δ -endotoxins disclosed and claimed herein can be used to identify and isolate other δ -endotoxins from a mixture of proteins. Specifically, antibodies may be raised to portions of the δ -endotoxins which are most constant and most distinct from other *B. thuringiensis* δ -endotoxins. These antibodies can then be used to specifically identify equivalent δ -endotoxins with the characteristic insecticidal activity by immunoprecipitation, enzyme linked immunoassay (ELISA), or Western blotting.

A further method for identifying the δ -endotoxins and genes of the subject invention is through the use of oligonucleotide probes. These probes are nucleotide sequences having a detectable label. As is well known in the art, if the probe molecule and nucleic acid sample hybridize by forming a strong bond between the two molecules, it can be reasonably assumed that the probe and sample are essentially identical. The probe's detectable label provides a means for determining in a known manner whether hybridization has occurred. Such a probe analysis provides a rapid method for identifying fomicidal β -endotoxin genes of the subject invention.

The nucleotide segments which are used as probes according to the invention can be synthesized by use of DNA synthesizers using standard procedures. In the use of the nucleotide segments as probes, the particular probe is labeled with any suitable label known to those skilled in the art, including radioactive and non-radioactive labels. Typical radioactive labels include ³²P, ¹²⁵I, ³⁵S, or the like. A probe labeled with a radioactive isotope can be constructed from a nucleotide sequence complementary to the DNA sample by a conventional nick translation reaction, using a DNAase and DNA polymerase. The probe and sample can then be combined in a hybridization buffer solution and held at an appropriate temperature until annealing occurs. Thereafter, the membrane is washed free of extraneous materials, leaving the sample and bound probe molecules typically detected and quantified by autoradiography and/or liquid scintillation counting.

Non-radioactive labels include, for example, ligands such as biotin or thyroxine, as well as enzymes such as hydrolases or peroxidases, or the various chemiluminescers such as luciferin, or fluorescent compounds like fluorescein and its derivatives. The probe may also be labeled at both ends with

different types of labels for ease of separation as, for example, by using an isotopic label at the end mentioned above and a biotin label at the other end.

Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid, and, as noted above, a certain degree of mismatch can be tolerated. Therefore, the probes of the subject invention include mutations (both single and multiple), deletions, insertions of the described sequences, and combinations thereof, wherein said mutations, insertions and deletions permit formation of stable hybrids with the target polynucleotide of interest. Mutations, insertions, and deletions can be produced in a given polynucleotide sequence in many ways, by methods currently known to an ordinarily skilled artisan, and perhaps by other methods which may become known in the future.

The potential variations in the probes listed is due, in part, to the redundancy of the genetic code, i.e., more than one coding nucleotide triple (codon) can be used for most of the amino acids used to make proteins. Therefore difficult nucleotide sequences can code for a particular amino acid. Thus, the amino acid sequences of the *B. thuringiensis* δ -endotoxins and peptides can be prepared by equivalent nucleotide sequences encoding the same amino acid sequence of the protein or peptide. Accordingly, the subject invention includes such equivalent nucleotide sequences. Also, inverse or completed sequences are an aspect of the subject invention and can be readily used by a person skilled in this art. In addition, it has been shown that proteins of identified structure and function may be constructed by changing the amino acid sequence if such changes do not alter the protein secondary structure (Kaiser and Kezdy, 1984). Thus, the subject invention includes mutants of the amino acid sequence depicted herein which do not alter the protein secondary structure, or if the structure is altered, the biological activity is substantially retained. Further, the invention also includes mutants of organisms hosting all or part of a δ -endotoxin encoding a gene of the invention. Such mutants can be made by techniques well known to persons skilled in the art. For example, UV irradiation can be used to prepare mutants of host organisms. Likewise, such mutants may include asprogenous host cells which also can be prepared by procedures well known in the art.

4.15 Ribozymes

Ribozymes are enzymatic RNA molecules which cleave particular mRNA species. In certain embodiments, the inventors contemplate the selection and utilization of ribozymes capable of cleaving the RNA segments of the present invention, and their use to reduce activity of target mRNAs in particular cell types or tissues.

Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in trans (and thus can cleave other RNA molecules) under physiological conditions. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of an enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved in RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over many technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the concentration of ribozyme necessary to affect a therapeutic treatment is lower than the of an antisense oligonucleotide. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme. Similar mismatches in antisense molecules do not prevent their action (Woelfel et al., 1992). Thus, the specificity of action of a ribozyme is greater than that of antisense oligonucleotide binding the same RNA site.

The enzymatic nucleic acid molecule may be formed in a hammerhead, hairpin, a hepatitis δ virus, group I intron or RNase PRNA (in association with an RNA guide sequence) or *Neurospora* VS RNA motif. Examples of hammerhead motifs are described by Rossi et al. (1992); examples of hairpin motifs are described by Hampel et al. (Eur. Pat. EP 0360257), Hampel and Triz (1989), Hampel et al. (1990) and Cech et al. (U.S. Pat. No. 5,631,359; an example of the hepatitis δ virus motif is described by Perrotta and Been (1992); an example of the RNaseP motif is described by Guerrier-Takada et al. (1983); *Neurospora* VS RNA ribozyme motif is described by Collins (Saville and Collins, 1990; Saville and Collins, 1991; Collins and Olive, 1993); and an example of the Group I intron is described by Cech et al. (U.S. Pat. No. 4,987,071). All that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule. Thus the ribozyme constructs need not be limited to specific motifs mentioned herein.

The invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target mRNA such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA or RNA vectors that are delivered to specific cells.

Small enzymatic nucleic acid motifs (e.g., of the hammerhead or the hairpin structure) may be used for exogenous delivery. The simple structure of these molecules increases the ability of the enzymatic nucleic acid to invade targeted regions of the mRNA structure. Alternatively, catalytic RNA molecules can be expressed within cells from eukaryotic promoters (e.g., Scanlon et al., 1991; Kashani-Sabet et al., 1992; Dropulic et al., 1992; Weerasinghe et al., 1991; Ojwang et al., 1992; Chen et al., 1992; Sarver et al., 1990). Those skilled in the art realize that any ribozyme can be expressed in eukaryotic cells from the appropriate DNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Draper et al., Int. Pat. Appl. Publ. No. WO 93/23569, and Sullivan et al., Int. Pat. Appl. Publ. No. WO 94/02595, both hereby incorporated in their totality by

reference herein; Ohkawa et al., 1992; Taira et al., 1991; Ventura et al., 1993).

Ribozymes may be added directly, or can be completed with cationic lipids, lipid complexes, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through injection, aerosol inhalation, infusion pump or stent, with or without their incorporation in biopolymers.

ribozymes may be designed as described in Draper et al. (Int. Pat. Appl. Publ. No. WO 93/23569), or Sullivan et al., (Int. Pat. Appl. Publ. No. WO 94/02595) and synthesized to be tested *in vitro* and *in vivo*, as described. Such ribozymes can also be optimized for delivery. While specific examples are provided, those in the art will recognize that equivalent RNA targets in other species can be utilized when necessary.

Hammerhead or hairpin ribozymes may be individually analyzed by computer folding (Jaeger et al., 1989) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Ribozymes of the hammerhead or hairpin motif may be designed to anneal to various sites in the mRNA message, and can be chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman et al. (1987) and in Scaringe et al. (1990) and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosboramidites at the 3'-end. Average stepwise coupling yields are typically >98%. Hairpin ribozymes may be synthesized in two parts and annealed to reconstruct an active ribozyme (Chowrira and Burke, 1992). Ribozymes may be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-o-methyl, 2'-H (for a review see Usman and Cedergren, 1992). Ribozymes may be purified by gel electrophoresis using general methods or by high pressure liquid chromatography and resuspended in water.

Ribozyme activity can be optimized by altering the length of the ribozyme binding arms, or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see e.g. Int. Pat. Appl. Publ. No. WO 92/07065; Perrault et al., 1990; Pieken et al., 1991; Usman and Cedergren, 1992; Int. Pat. Appl. Publ. No. WO 93/15187; Int. Pat. Appl. Publ. No. WO 91/03162; Eur. Pat. Appl. Publ. No. 92110298.4; U.S. Pat. No. 5,334,711; and Int. Pat. Appl. Publ. No. WO 94/13688, which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules), modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan et al. (Int. Pat. Appl. Publ. No. WO 94/02595) describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. For some indications, ribozymes may be directly delivered *ex vivo* to

cells or tissues with or without the aforementioned vehicles. Alternatively, the RNA/vehicle combination may be locally delivered by direct inhalation, by direct injection or by use of a catheter, infusion pump or stent. Other routes of delivery include, but are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Sullivan et al. (Int. Pat. Appl. Publ. No. WO 94/02595) and Draper et al. (Int. Pat. Appl. Publ. No. WO 93/23659) which have been incorporated by reference herein.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters may also be used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990; Gao and Huang, 1993; Lieber et al., 1993; Zhou et al., 1990). Ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Saber et al., 1992; Ojwang et al., 1992; Chen et al., 1992; Yu et al., 1993; L'Huillier et al., 1992; Lisiewicz et al., 1993). Such transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated vectors), or viral RNA vectors (such as retroviral, semliki forest virus, sindbis virus vectors).

Ribozymes of this invention may be used as diagnostic tools to examine genetic drift and mutations within cell lines or cell types. They can also be used to assess levels of the target RNA molecule. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple ribozymes described in this invention, one may map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in particular cells or cell types.

5.0 EXAMPLES

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

5.1 Example 1

Three-dimensional Structure Cry3Bb

The three-dimensional structure of Cry3Bb was determined by X-ray crystallography. Crystallization of Cry3Bb

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and X-ray diffraction data collection were performed as described by Cody et al. (1992). The crystal structure of Cry3Bb was refined to a residual R factor of 18.0% using data collected to 2.4 Å resolution. The crystals belong to the space group C222, with unit cell dimensions $a=122.44$, $b=131.81$, and $c=105.37$ Å and contain one molecule in the asymmetric unit. Atomic coordinates for Cry3Bb are described in Example 31 and listed in Section 9.

The structure of Cry3Bb is similar to that of Cry3A (Li et al., 1991). It consists of 5825 protein atoms from 588 residues (amino acids 64–652) forming three discrete domains (FIG. 1). A total of 251 water molecules have been identified in the Cry3Bb structure (FIG. 2). Domain 1 (residues 64–294) is a seven helical bundle formed by six helices twisted around the central helix, $\alpha 5$ (FIG. 3). The amino acids forming each helix are listed in FIG. 4. Domain 2 (residues 295–502) contains three antiparallel β -sheets (FIG. 5A and FIG. 5B). Sheets 1 and 2, each composed of 4 β strands, form the distinctive “Greek key” motif. The outer surface of sheet 3, composed of 3 β strands, makes contact with helix $\alpha 7$ of domain 1. FIG. 6 lists the amino acids comprising each β strand in domain 2. A small α helix, $\alpha 8$ which follows β strand 1, is also included in domain 2. Domain 3 (residues 503–652) has a “jelly roll” β -barrel topology which has a hydrophobic core and is nearly parallel to the α and perpendicular to the c axes of the lattice (FIG. 7A and FIG. 7B). The amino acids comprising each β strand of domains 3 are listed in FIG. 8.

The monomers of Cry3Bb in the crystal form a dimeric quaternary structure along a two-fold axis parallel to the α axis (FIG. 9A and FIG. 9B). helix $\alpha 6$ lies in a cleft formed by the interface of domain 1 and domains 1 and 3 of its symmetry related molecule. There are numerous close hydrogen bonding contacts along this surface, confirming the structural stability of the dimer.

5.2 Example 2

Precipitation of Cry3Bb.60

B. thuringiensis EG7231 was grown through sporulation in C2 medium with chloramphenicol (Cml) selection. The solids from this culture were recovered by centrifugation and washed with water. The toxin was purified by recrystallization from 4.0M NaBr (Cody et al., 1992). The purified Cry3Bb was solubilized in 10 ml of 50 mM KOH/100 mg Cry3Bb and buffered to pH 9.0 with 100 mM CAPS (pH 9.0). The soluble toxin was treated with trypsin at a weight ratio of 50 mg toxin to 1 mg trypsin. After 20 min of trypsin digestion the predominant protein visualized by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was 60 kDa. Further digestion of the 60-kDa toxin was not observed. FIG. 4 illustrates the Coomassie-stained Cry3Bb and Cry3Bb.60 following SDS-PAGE.

5.3 Example 3

Purification and Sequence of Cry3Bb.60

Cry3Bb.60 was electrophoretically purified by SDS-PAGE and electroblotted to Immobilon-® (Millipore) membrane by semi-dry transfer at 15V for 30 min. The membrane was then washed twice with water and stained with 0.025% R-250, 40% methanol. To reduce the background, the blot was destained with 50% methanol until the stained protein bands were visible. The blot was then air dried, and the stained Cry3Bb.60 band was cut out of the membrane. This band was sent to the Tufts University Sequencing Labora-

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tory (Boston, MA) for N-terminal sequencing. The experimentally-determined N-terminal amino acid sequence is shown in Table 7 beside the known amino acid sequence starting at amino acid residue 160.

TABLE 7

AMINO ACID SEQUENCE OF THE N-TERMINUS OF CRY3BB.60
AND COMPARISON TO THE KNOWN SEQUENCE OF CRY3BB

Deduced Sequence	Known Sequence	Residue #
S	S	160
K	K	161
R	R	262
S	S	163
Q	Q	164
D	D	165
R	R	166

5.4 Example 4

Bioactivity of Cry3Bb.60

Cry3Bb was prepared for bioassay by solubilization in a minimal amount of 50 mM KOH, 10 ml per 100 mg toxin, and buffered to pH 9.0 with 100 mM CAPS, pH 9.0. Cry3Bb.60 was prepared as described in Example 1. Both preparations were kept at room temperature 12 to 16 hours prior to bioassay. After seven days the mortality of the population was determined and analyzed to determine the lethal concentration of each toxin. These results are summarized in Table 8.

TABLE 8

BIOACTIVITY OF CRY3BB AND CRY3BB.60
AGAINST THE SOUTHERN CORN ROOTWORM
(*DIABLOTICA UNDECIMPUNCTATA*)

	LC ₅₀ mg/well	95% C.I.
Cry3Bb	24.09	15–39
Cry3Bb.60	6.72	5.25–8.4

5.5 Example 5

Ion-channel Formation by Cry3Bb AND CryB2.60

Cry3Bb.60 and Cry3Bb were evaluated for their ability to form ion channels in planar lipid bilayers. Bilayers of phosphatidylcholine were formed on Teflon® supports over a 0.7-mm hole. A bathing solution of 3.5 ml 100 mM KOH, 10 mM CaCl₂, 100 mM CAPS (pH 9.5) was placed on either side of the Teflon® partition. The toxin was added to one side of the partition and a voltage of 60 mV was imposed across the phosphatidylcholine bilayer. Any leakage of ions through the membrane was amplified and recorded. An analysis of the frequency of the conductances created by either Cry3Bb or Cry3Bb.60 are illustrated in FIG. 5A and FIG. 5B. Cry3Bb.60 readily formed ion channels whereas Cry3Bb rarely formed channels.

5.6 Example 6

Formation of High Molecular-weight Oligomers

Individual molecules of Cry3Bb or Cry3Bb.60 form a complex with another like molecule. The ability of Cry3Bb to form an oligomer is not reproducibly apparent. The complex cannot be repeatedly observed to form under

nondenaturing conditions. Cry3Bb.60 formed a significantly greater amount of a higher molecular-weight complex (≥ 120 kDa) with other Cry3Bb.60 molecules. Oligomers of Cry3Bb are demonstrated by the intensity of the Coomassie-stained SDS polyacrylamide gel. Oligomerization is visualized on SDS-PAGE by not heating samples prior to loading on the gel to retain some nondenatured toxin. These data suggest that Cry3Bb.60 more readily forms the higher order complex than Cry3Bb alone. Oligomerization is also observed by studying the conductance produced by these molecules and the time-dependent increase in conductance. This change in conductance can be attributed to oligomerization of the toxin.

5.7 Example 7

Design Method 1: Identification and Alteration of Protease-sensitive Sites and Proteolytic Processing

It has been reported in the literature that treatment of Cry3A toxin protein with trypsin, an enzyme that cleaves proteins on the carboxyl side of available lysine and arginine residues, yields a stable cleavage product of 55 kDa from the 67 kDa native protein (Carroll et al., 1989). N-terminal sequencing of the 55 kDa product showed cleavage occurs at amino acid residue R158. The truncated Cry3A protein was found to retain the same level of insecticidal activity as the native protein. Cry3Bb toxin protein was also treated with trypsin. After digestion, the protein size decreased from 68 kDa, the molecular weight of the native Cry3Bb toxin, to 60 kDa. No further digestion was observed. N-terminal sequencing revealed the trypsin cleavage site of the truncated toxin (Cry3Bb.60) to be amino acid R159 in $\alpha 3,4$ of Cry3Bb. Unexpectedly, the bioactivity of the truncated Cry3Bb toxin was found to increase.

Using this method, protease digestion of a *B. thuringiensis* toxin protein, a proteolytically sensitive site was identified on Cry3Bb, and a more highly active form of the protein (Cry3Bb.60) was identified. Modifications to this proteolytically-sensitive site by introducing an additional protease recognition site also resulted in the isolation of a biologically more active protein. It is also possible that removal of other protease-sensitive site(s) may improve activity. Proteolytically sensitive regions, once identified, may be modified or utilized to produce biologically more active toxins.

5.7.1 Cry3Bb.60

Treatment of solubilized Cry3Bb toxin protein with trypsin results in the isolation of a stable, truncated Cry3Bb toxin protein with a molecular weight of 60 kDa (Cry3Bb.60). N-terminal sequencing of Cry3Bb.60 shows the trypsin-sensitive site to be R159 in $\alpha 3,4$ of the native toxin. Trypsin digestion results in the removal of helices 1–3 from their native Cry3Bb but also increases the activity of the toxin against SCRW larvae approximately four-fold.

Cry3Bb.60 is a unique toxin with enhanced insecticidal use over the parent Cry3Bb. Improved biological activity, is only one parameter that distinguishes it as a new toxin. Aside from the reduced size, Cry3Bb.60 is also a more soluble protein. Cry3Bb precipitates from solution at pH 6.5 while Cry3Bb.60 remains in solution from pH 4.5 to pH 12. Cry3Bb.60 also forms ion channels with greater frequency than Cry3Bb.

Cry3Bb.60 is produced by either the proteolytic removal of the first 159 amino acid residues, or the in vivo production of this toxin, by bacteria or plants expressing the gene for Cry3Bb.60, that is, the Cry3Bb gene without the first 483 nucleotides.

In conclusion, Cry3Bb.60 is distinct from Cry3Bb in several important ways: enhanced insecticidal activity; enhanced range of solubility; enhanced ability to form channels; and reduced size.

5.7.2 EG11221

Semi-random mutagenesis of the trypsin-sensitive $\alpha 3,4$ region of Cry3Bb resulted in the isolation of Cry3Bb.11221, a designed Cry3Bb protein that exhibits over a 6-fold increase in activity against SCRW larvae compared to WT. Cry3Bb.11221 has 4 amino acid changes in the $\alpha 3,4$ region. One of these changes, L158R, introduces an additional trypsin site adjacent to R159, the proteolytically sensitive site used to produce Cry3Bb.60 (example 4.1.1). Cry3Bb.11221 is produced by *B. thuringiensis* as a full length toxin protein but is presumably digested by insect gut protease to the same size as Cry3Bb.60 (see Cry3A results from Carroll et al., 1989). The additional protease recognition site may make the $\alpha 3,4$ region even more sensitive to digestion, thereby increasing activity.

5.8 Example 8

Design Method 2: Determination and Manipulation of Bound Water

There are several ways that water molecules can associate with a protein, including surface water that is easily removed and bound water that is more difficult to extract (Dunitz, 1994; Zhang and Matthews, 1994). The function of bound water has been the subject of significant academic extrapolation, but the precise function has little experimental validation. Some of the most interesting bound or structural water is the water that precipitates in the protein structure from inside the protein itself.

The occupation of a site by a water molecule can indicate a stable pocket within a protein or a looseness of packing created by water-mediated salt bridges and hydrogen bonding to water. This can reduce the degree of bonding between amino acids, possibly making the region more flexible. A different amino acid sequence around that same site could result in better packaging, collapsing the pocket around polar or charged amino acids. This may result in decreased flexibility. Therefore, the degree of hydration of a region of a protein may determine the flexibility or mobility of that region, and manipulation of the hydration may alter the flexibility. Methods of increasing the hydration of a water-exposed region include increasing the number of hydrophobic residues along that surface. It is taught in the art that exposed hydrophobic residues require significantly more water to hydrate than hydrophilic residues (CRC Handbook of Chemistry and Physics, CRC Press, Inc.). It is not taught, however, that by doing this, improvements to the biological activity of a protein can be achieved.

Structural water has not previously been identified in *B. thuringiensis* δ -endotoxins including Cry3Bb. Furthermore, there are no reports of the function to this structural water in δ -endotoxins or bacterial toxins. In the analysis of Cry3Bb, it was observed that a collection of water molecules are located around $\alpha 3,4$, a site defined by the inventors as important for improvement of bioactivity. The loop $\alpha 3,4$ region is surface exposed and may define a hinge in the protein permitting either removal or movement of the first three helices of domain 1. The hydration found around this region may impart flexibility and mobility to this loop. The observation of structural water all the $\alpha 3,4$ site provided an analytical tool for further structure analysis. If this important

site is surrounded by water, then other important sites may also be completely or partially surrounded by water. Using this insight, structural water surrounding helices 5 and 6 was then identified. This structural water forms a column through the protein, effectively separating helices 5 and 6 from the rest of the molecule. The structure of Cry3A and Cry3Bb suggest that helices 5 and 6 are tightly associated, bound together by Van de Waals interactions. Alone, helix 5 from Cry3A, although insufficient for biological activity, has been demonstrated to have the ability to form ion channels in an artificial membrane (Gazit and Shai, 1993). The ion channels formed by elix 5 are 10-fold smaller than the channels of the full length toxin suggesting that significantly more toxin structure is required for the full-sized ion channels. In Cry3Bb, helix 5 as part of a cluster of a helices (domain 1) has been found to form ion channels (Von Tersch et al., 1994). Unpublished experimental observations by the inventors demonstrate that helix 6 also crossed the biological membrane. Helices 5 and 6, therefore, are the putative channel-forming helices necessary for toxicity.

The hydration around these helices may indicate that flexibility of this region is necessary for toxicity. It is conceivable, therefore, that if it were possible to improve the hydration around helices 5 and 6, one could create a better toxin protein. Care must be taken, however, to avoid creating continuous hydrophobic surfaces between helices 5-6 and any other part of the protein which could, by hydrophobic interactions, act to restrict movement of the mobile helices. The mobility of helices 5 and 6 may also depend on the flexibility of the loops attached to them as well as on other regions of the Cry3Bb molecule, particularly in domain 1, which may undergo conformational changes to allow insertion of the 2 helices into the membrane. Altering the hydration of these regions of the protein may also affect its bioactivity.

5.8.1 Cry3Bb.11032

A collection of bound water residues indicated the relative flexibility of the α 3,4 region. The flexibility of this loop can be increased by increasing the hydration of the region by substituting relatively hydrophobic residues for the exposed hydrophilic residues. An example of an improved, designed protein having this type of substitution is Cry3Bb.11032. Cry3Bb.11032 has the amino acid change D165G; glycine is more hydrophobic than aspartate (Kyte and Doolittle hydrophobicity score of -4.0 vs. -3.5 for aspartate). Cry3Bb.11032 is approximately 3 times more active than WT Cry3Bb.

5.8.2 Cry3Bb.11051

To increase the hydration of the α 4,5 region of Cry3Bb, glycine was substituted for the surface exposed residue K189. Glycine is more hydrophobic than lysine (Kyte and Doolittle hydrophobicity score of -0.4 vs. -3.9 for lysine) and may result in an increase in bound water. The increase in bound water may impart greater flexibility to the loop region which precedes the channel-forming helix, α 5. The designed Cry3Bb protein with the K189G change, Cry3Bb.11051, exhibits a 3-fold increase in activity compared to WT Cry3Bb.

5.8.3 Alterations to α 7, β 1 (Cry3Bb.11241 and 11242)

Amino acid changes made in the surface-exposed loop connecting α -helix 7 and β -strand (α 7, β 1) resulted in the identification of 2 altered Cry3 Bb proteins with increased

bioactivities, Cry3Bb.11241 and Cry3Bb.11242. Analysis of the hydropathy index of 2 of these proteins over the 20 amino acid sequence 281-300, inclusive of the α 7, β 1 region, reveal that the amino acid substitutions in these proteins have made the α 7, β 1 region much more hydrophobic. The grand average of hydropathy value (GRAVY) was determined for each protein sequence using the PC/GENE® (IntelliGenetics, Inc., Mountain View, Calif., release 6.85) protein sequence analysis computer program, SOAP, and a 7 amino acid interval. The SOAP program is based on the method of Kyte and Doolittle (1982). The increase in hydrophobicity of the α 7, β 1 region for each protein may increase the hydration of the loop and therefore, the flexibility. The altered proteins, their respective amino acid changes, fold-increases over WT bioactivity, and GRAVY values are listed in Table 9.

TABLE 9

HYDROPATHY VALUES FOR THE α 7, β 1 REGION OF CRY3BB AND 2 DESIGNED CRY3BB PROTEINS SHOWING INCREASED SCRW BIOACTIVITY

Cry3Bb* Protein	Amino Acid Changes	Fold Increase in Bioactivity Over WT	GRAVY (Amino Acids 281-300)
wildtype	—	—	4.50
Cry3Bb.11241	Y287F, D288N, R290L	2.6x	10.70
Cry3Bb.11242	R290V	2.5x	8.85

5.8.4 Alterations of β 1, α 8 (Cry3Bb.11228, Cry3Bb.11229, Cry3Bb.11230, Cry3Bb.11233, Cry3Bb.11236, Cry3Bb.11237, Cry3Bb.11238, and Cry3Bb.11239)

The surface-exposed loop between β -strand 1 and α -helix 8 (β 1, α 8) defines the boundary between domains 1 and 2 of Cry3Bb. The introduction of semi-random amino acid changes to this region resulted in the identification of several altered Cry3Bb proteins with increased bioactivity. Hydropathy index analysis of the amino acid substitutions found in the altered proteins shows that the changes have made the exposed region more hydrophobic which may result in increased hydration and flexibility. Table 10 lists the altered proteins, their respective amino acid changes and fold increases over WT Cry3Bb and the grand average of hydropathy value (GRAVY) determined using the PC/GENE® (IntelliGenetics, Inc., Mountain View, Calif., release 6.85) protein sequence analysis program, SOAP, over the 20 amino acid sequence 305-324 inclusive of β 1, α 8, using a 7 amino acid interval.

TABLE 10

HYDROPATHY VALUES FOR THE β 1, μ 8 REGION OF CRY3BB AND 8 DESIGNED CRY3BB* PROTEINS SHOWING INCREASED SCRW BIOACTIVITY

Cry3Bb* Protein	Amino Acid Changes	Fold Increase in Bioactivity Over Wild Type	GRAVY (Amino Acids 305-324)
wildtype	—	—	0.85
Cry3Bb.11228	S311I, N313T, E317K	4.1x	4.35
Cry3Bb.11229	S311T, E317K, Y318C	2.5x	2.60

TABLE 10-continued

HYDROPATHY VALUES FOR THE L β 1, μ 8 REGION OF CRY3BB AND 8 DESIGNED CRY3BB* PROTEINS SHOWING INCREASED SCRW BIOACTIVITY			
Cry3Bb* Protein	Amino Acid Changes	Fold Increase in Bioactivity Over Wild Type	GRAVY (Amino Acids 305-324)
Cry3Bb.11230	S311A, L312V, Q316W	4.7x	3.65
Cry3Bb.11233	S311A, Q316D	2.2x	2.15
Cry3Bb.11236	S311I	3.1x	3.50
Cry3Bb.11237	S311I, N313H	5.4x	3.65
Cry3Bb.11238	N313V, T314N, Q316M, B317V	2.6x	9.85
Cry3Bb.11239	N313R, L315P, Q316L, E317A	2.8x	3.95

5.8.5 Cry3Bb.11227, Cry3Bb.11241 and Cry3Bb.11242

Amino acid Q238, located in helix 6 of Cry3Bb, has been identified as a residue that, by its large size and hydrogen bonding to R290, blocks complete hydration of the space between helix 6 and helix 4. Substitution of R290 with amino acids that do not form hydrogen bonds or that have side chains that can not span the physical distance to hydrogen bond with Q238 may result in increased hydration around Q238. Q238, unable to hydrogen bond to R290, may now bind water. This may increase the flexibility of the channel-forming region. Designed proteins Cry3Bb.11227 (R290N), Cry3Bb.11241 (R290L) and Cry3Bb.11242 (R290V) show increased activities of approximately 2-fold, 2.6-fold and 2.5-fold, respectively, against SCRW larvae compared to WT.

5.9 Example 9

Design Method 3: Manipulation of Hydrogen Bonds Around Mobile Regions

Mobility of regions of a protein may be required for activity. The mobility of the α 5,6 region, the putative channel-forming region of Cry3Bb, may be improved by decreasing the number of hydrogen bonds, including salt bridges (hydrogen bonds between oppositely charged amino acid side chains), between helices 5-6 and any other part of the molecule or dimer structure. These hydrogen bonds may impede the movement of the two helices. Decreasing the number of hydrogen bonds and salt bridges may improve biological activity. Replacement of hydrogen-bonding amino acids with hydrophobic residues must be done with caution to avoid creating continuous hydrophobic surfaces between helices 5-6 and any other part of the dimer. This may decrease mobility by increasing hydrophobic surface interactions.

5.9.1 Cry3Bb.11222 and Cry3Bb.11223

Tyr230 is located on helix 6 and, in the quaternary dimer structure of Cry3Bb, this amino acid is coordinated with Tyr230 from the adjacent molecule. Three hydrogen bonds are formed between the two helices 6 in the two monomers because of this single amino acid. In order to improve the flexibility of helices 5-6, the helices theoretically capable of penetrating the membrane and forming an ion channel, the hydrogen bonds across the dimer were removed by changing this amino acid and a corresponding increase in biological

activity was observed. The designed Cry3Bb proteins, Cry3Bb.11222 and Cry3Bb.EG11223, show a 4-fold and 2.8-fold increase in SCRW activity, respectively, compared to WT.

5.9.2 Cry3Bb.11051

Designed Cry3Bb protein Cry3Bb.11051 has amino acid change K189G in α 4,5 of domain 1. In the WT Cry3Bb structure, the exposed side chain of K189 is close enough to the exposed side change of E123, located in I=2b,3, to form hydrogen bonds. Substitution of K189 with glycine, as found in this position in Cry3A, removes the possibility of hydrogen bond formation at this site and results in a protein with a bioactivity three-fold greater than WT Cry3Bb.

5.9.3 Cry3Bb.11227, Cry3Bb.11241 and Cry3Bb.11242

Amino acid Q238, located in helix 6 of Cry3Bb, has been identified as a residue that, by its large size and hydrogen bonding to R290, blocks complete hydration of the space between helix 6 and helix 4. Substitution of R290 with amino acids that do not form hydrogen bonds or that have side chains that can not span the physical distance to hydrogen bond with Q238 may increase the flexibility of the channel-forming region. Designed proteins Cry3Bb.11227 (R290N), Cry3Bb.11241 (R290L), and Cry3Bb.11242 (R290V) show increased activities of approximately 2-fold, 2.6-fold and 2.5-fold, respectively, against SCRW larvae compared to WT.

5.10 Example 10

Design Method 4: Loop Analysis and Loop Design Around Flexible Helices

Loop regions of a protein structure may be involved in numerous functions of the protein including, but not limited to, channel formation, quaternary structure formation and maintenance, and receptor binding. Cry3Bb is a channel-forming protein. The availability of the ion channel-forming helices of δ -endotoxins to move into the bilayer depend upon the absence of forces that hinder the process. One of the forces possibly limiting this process is the state hindrance of amino acid side chains in loop regions around the critical helices. The literature suggests that in at least one other bacterial toxin, not a B. thuringiensis toxin, the toxin molecule opens up or, in scientific terms, loses some of the quaternary structure to expose a membrane-active region (Cramer et al., 1990). This literature does not teach how to improve the probability of this event occurring and it is not known if B. thuringiensis toxins use this same process to penetrate the membrane. Reducing the steric hindrance of the amino acid side chains in these critical regions by reducing size or altering side chain positioning with the corresponding increase in biological activity was the invention step.

5.10.1 Analysis of the Loop Between Helices 3 and 4 (Cry3Bb.11032)

The inventors have discovered that the first three helices of domain one could be cleaved from the rest of the toxicity by proteolytic digestion of the loop between helices α 3 and α 4 (Cry3Bb.60). Initial efforts to truncate the cry3Bb gene to produce this shortened, though more active Cry3Bb molecule, failed. For unknown reasons, B. thuringiensis failed to synthesize this 60-kDa molecule. It was then reasoned that perhaps the first three helices of domain 1 did

not have to be proteolytically removed, or equivalently, the protein did not have to be synthesized in this truncated form to take advantage of the Cry3Bb.60 design. It was observed that the protein Cry3A had a small amino acid near the $\alpha 3,4$ that might impart greater flexibility in the loop region thereby permitting the first three helices of domain 1 to move out of the way, exposing the membrane-active region. By designing a Cry3Bb molecule with a glycine residue near this loop, the steric hindrance of residues in the loop might be lessened. The redesigned protein, Cry3Bb.11032, has the amino acid change D165G, which replaces the larger aspartate residue (average mass of 115.09) with the smallest amino acid, glycine (average mass of 57.05). The activity of Cry3Bb.11032 is approximately 3-fold greater than that of the WT protein. In this way, the loop between helices $\alpha 3$ and $\alpha 4$ was rationally redesigned with a corresponding increase in the biological activity.

5.10.2 Cry3Bb.11051

The loop region concentrating helices $\alpha 4$ and $\alpha 5$ in Cry3Bb must be flexible so that the channel-forming helices $\alpha 5$ - $\alpha 6$ can penetrate into the membrane. It was noticed that Cry3A has a glycine residue in the middle of this loop that may impart greater flexibility. The corresponding change, K189G, was made in Cry3Bb and the resulting, designed protein, Cry3Bb.11051, exhibits a 3-fold increase in activity against SCRW larvae compare to WT Cry3Bb.

5.10.3 Analysis of the Loop Between β -strand 1 and Helix 8 (Cry3Bb.11228, Cry3Bb.11229, Cry3Bb.11230, Cry3Bb.11232, Cry3Bb.11233, Cry3Bb.11236, Cry3Bb.11237, Cry3Bb.11238, and Cry3Bb.11239)

The loop region located between β strand 1 of domain 2 and α helix 8 in domain 2 is very close to the loop between α helices 6 and 7 in domain 1. Some of the amino acids side chains of $1\beta 1, \alpha 8$ appear as though they may sterically impede movement of $1\alpha 6, 7$. Since the $1\alpha 7$, must be flexible for the channel-forming helices $\alpha 5$ - $\alpha 6$ to insert into the membrane, it was thought that re-engineering this loop may change the positioning of the side chains resulting in less steric hindrance. This was accomplished creating proteins with increased biological activities ranging from 2.2 to 5.4 times greater than WT. These designed toxin proteins and their amino acid changes are listed in Table 2 as Cry3Bb.11228, Cry3Bb.11229, Cry3Bb.11230, Cry3Bb.11232, Cry3Bb.11233, Cry3Bb.11236, Cry3Bb.11237, Cry3Bb.11238, and Cry3Bb.11239.

5.10.4 Analysis of the Loop Between Helix 7 and β -strand 1 (Cry3Bb.11227, Cry3Bb.11234, Cry3Bb.11241, Cry3Bb.11242, and Cry3Bb.11036)

If Cry3Bb is similar to a bacterial toxin which must open up to expose a membrane active region for toxicity, it is possible that other helices in addition to the channel-forming helices must also change positions. It was reasoned that, if helices $\alpha 5$ - $\alpha 6$ insert into the membrane, then helix $\alpha 7$ may have to change positions also. It was shown in example 4.4.3 that increasing flexibility between helix $\alpha 6$ and $\alpha 7$ can increase activity, greater flexibility in the loop following helix $\alpha 7, 1\alpha 7, \beta 1$ may also increase bioactivity. Alterations to the $1\alpha 7, \beta 1$ region of Cry3Bb resulted in the isolation of several proteins with increased activities ranging from 1.9 to 4.3 times greater than WT. These designed proteins are listed in Table 7 as Cry3Bb.11227, Cry3Bb.11234, Cry3Bb.11241, Cry3Bb.11242, and Cry3Bb.11036.

5.11 Example 11

Design Methods 5: Loop Design Around β Strands and β Sheets

Loop regions of a protein structure may be involved in numerous functions of the protein including, but not limited to, channel formation, quaternary structure formation and maintenance, and receptor binding. A binding surface is often defined by a number of loops, as is the case with immunoglobulin G (IgG) (see Branden and Tooze, 1991, for review). What can not be determined at this point, however, is what loops will be important for receptor interactions just by looking at the structure of the protein in question. Since a receptor has not been identified for Cry3Bb, it is not even possible to compare the structure of Cry3Bb with other proteins that have the same receptor for structural similarities. To identify Cry3Bb loops that contribute to receptor interactions, random mutagenesis was performed on surface-exposed loops.

As each loop was altered, the profile of the overall bioactivities of the resultant proteins were examined and compared. The loops, especially in domain 2 which appears to be unnecessary for channel activity, fall into two categories: (1) loops that could be altered without much change in the level of bioactivity of the resultant proteins and (2) loops where alterations resulted in overall loss of resultant protein bioactivity. Using this design method, it is possible to identify several loops important for activity.

5.11.1 Analysis of Loop $\beta 2,3$

Semi-random mutagenesis of the loop region between β strands 2 and 3 resulted in the production of structurally stable toxin proteins with significantly reduced activities against SCRW larvae. The $1\beta 2,3$ region is highly sensitive to amino acid changes indicating that specific amino acids or amino acid sequences are necessary for toxin protein activity. It is conceivable, therefore, that specific changes in the $1\beta 2,3$ region will increase the binding and, therefore, the activity of the redesigned toxin protein.

5.11.2 Analysis of Loop $\beta 6,7$

Semi-random mutations, introduced to the loop region between β strands 6 and 7 resulted in structurally stable proteins with an overall loss of SCRW bioactivity. The $1\beta 6,7$ region is highly sensitive to amino acid changes indicating that specific amino acids or amino acid sequences are necessary for toxin protein activity. It is conceivable, therefore, that specific changes in the $1\beta 6,7$ region will increase the binding and, therefore, the activity of the redesigned toxin protein.

5.11.3 Analysis of Loop $\beta 10,11$

Random mutations to the loop region between β strands 10 and 11 resulted in proteins having an overall loss of SCRW bioactivity. Loop $\beta 10,11$ is structurally close to and interacts with loops $\beta 2,3$ and $\beta 6,7$. Specific changes to individual residues within the $1\beta 10,11$ region may also result in increased interaction with the insect membrane, increasing the bioactivity of the toxin protein.

5.11.4 Cry3Bb.11095

Loops $\beta 2,3$, $\beta 6,7$ and $\beta 10,11$ have been identified as important for bioactivity for Cry3Bb. The 3 loops are surface-exposed and structurally close together. Amino acid Q348 in the WT structure, located in β -strand 2 just prior to

1 β 2,3 do not form any intramolecular contacts. However, replacing Q348 with arginine (Q348R) results in the formation of 2 new hydrogen-bonds between R348 and the backbone carbonyls of R487 and R488, both located in 1 β 10,11. The new hydrogen bonds may act to stabilize the structure formed by the 3 loops. The designed protein carrying this change, Cry3Bb.11095, is 4.6-fold more active than WT Cry3Bb.

5.12 Example 12

Design Method 6: Identification and Re-design of Complex Electrostatic Surfaces

Interactions of proteins include hydrophobic interactions (e.g., Van der Waals forces), hydrophilic interactions, including those between opposing charges on amino acid side chains (salt bridges), and hydrogen bonding. Very little is known about δ -endotoxin and receptor interactions. Currently, there are not literature reports identifying the types of interactions that predominate between *B. thuringiensis* toxins and receptors.

Experimentally, however, it is important to increase the strength of the *B. thuringiensis* toxin-receptor interaction and not permit the precise determination of the chemical interaction to stand in the way of improving it. To accomplish this, the electrostatic surface of Cry3Bb was defined by solving the Poisson-Boltzman distribution around the molecule. Once this electrically defined surface was solved, it could then be inspected for regions of greatest diversity. It was reasoned that these electrostatically diverse regions would have the greatest probability of participating in the specific interactions between the *B. thuringiensis* toxin proteins and the receptor, rather than more general and non-specific interactions. Therefore, these regions were chosen for redesign, continuing to increase the electrostatic diversity of the regions. In addition, examination of the electrostatic interaction around the putative channel forming region of the toxin created insights for redesign. This includes identification of an electropositive residue in an otherwise negatively charged conduit (see example 4.6.1).

5.12.1 R290 (Cry3Bb.11227, Cry3Bb.11241, and Cry3Bb.11242)

Examination of the Cry3Bb dimer interfaces along the domain 1 axis suggested that a pore or conduit for cations might be formed between the monomers. Electrostatic examination of this axis lent additional credibility to this suggestion. In fact, the hypothetical conduit is primarily negatively charged, an observation consistent with the biophysical analysis of cation-selective, δ -endotoxin channels. If a cation channel were formed along the axis of the dimer, then the cation could move between the monomers relatively easily with only one significant hurdle. A positively charged arginine residue (R290) lies in the otherwise negatively charged conduit. This residue could impede the cation movement through be channel. Based on this analysis, R290 was changed to unchanged residues. The bioactivity of redesigned proteins Cry3Bb.11227 (R290N), Cry3Bb.11241 (R290L) and Cry3Bb.11242 (R290V) was improved approximately 2-fold, 2.6-fold and 2.5-fold, respectively.

5.12.2 Cry3Bb.60

Trypsin digestion of solubilized Cry3Bb yields a stable, truncated protein with a molecular weight of 60 kDa (Cry3Bb.60). Trypsin digestion occurs on the carboxyl side of residue R159, effectively removing helices 1 through 3

from the native Cry3Bb structure. The cleavage of the first 3 helices exposes an electrostatic surface different than those found in the native structure. The new surface has a combination of hydrophobic, polar and charged characteristics that may play a role in membrane interactions. The bioactivity of Cry3Bb.60 is 3.6-fold greater than that of WT Cry3Bb.

5.13 Example 13

Design Method 7: Identification and Removal of Metal Binding Sites

The literature teaches that the in vitro behavior of *B. thuringiensis* toxins can be increased by chelating divalent cations from the experimental system (Crawford and Harvey 1988). It was not known, however, how these divalent cations inhibited the in vitro activity. Crawford and Harvey (1988) demonstrated that the short circuit current across the midgut was more severely inhibited by *B. thuringiensis* in the presence of EDTA, a chelator of divalent ions, than in the absence of this agent, thus suggesting that this step in the mode of action of *B. thuringiensis* could be potentiated by removing divalent ions. Similar observations were made using black-lipid membranes and measuring an increase in the current created by the δ -endotoxins in the presence of EDTA to chelate divalent ions. There were at least three possible explanations for these observations. The first explanation could be that the divalent ions are too large to move through a ion channel more suitable for monovalent ions, thereby blocking the channel. Second, the divalent ions may cover the protein in the very general way, thereby buffering the charge interactions required for toxin membrane interaction and limiting ion channel activity. The third possibility is that a specific metal binding site exists on the protein and, when occupied by divalent ions, the performance of the ion channel is impaired. Although the literature could not differentiate the value of one possibility over another, the third possibility led to an analysis of the Cry3Bb structure searching for a specific metal binding site that might alter the probability that a toxin could form an ion channel.

5.13.1 H231 (Cry3Bb.11222, Cry3Bb.11224, Cry3Bb.11225, and Cry3Bb.11226)

A putative metal binding site is formed in the Cry3Bb dimer structure by the H231 residues of each monomer. The H231 residues, located in helix α 6, lie adjacent to each other and close to the axis of symmetry of the dimer. Removal of this site by replacement of histidine with other amino acids was evaluated by the absence of EDTA-dependent ion channel activity. The bioactivities of the designed toxin proteins, Cry3Bb.11222, Cry3Bb.11224, Cry3Bb.11225 and Cry3Bb.11226, are increased 4-, 5-, 3.6- and 3-fold, respectively, over that of WT Cry3Bb. Their respective amino acid changes are listed in Table 2.

5.14 Example 14

Design Method 8: Alteration of Quaternary Structure

Cry3Bb can exist in solution as a dimer similar to a related protein, Cry3A (Walters et al., 1992). However, the importance of the dimer to biological activity is not known because the toxin as a monomer or as a higher order structure has not been seriously evaluated. It is assumed that specific amino acid residues contribute to the formation and stability of the quaternary structure. Once a contributing

residue is identified, alterations can be made to diminish or enhance the effect of that residue thereby affecting the interaction between monomers. Channel activity is a useful way, but by no means the only way, to assess quaternary structure of Cry3Bb and its derivatives. It has been observed that Cry3Bb creates gated conductances in membranes that grow in size with time, ultimately resulting in large pores in the membrane (the channel activity of WT Cry3Bb is described in Section 12.1). It also has been observed that Cry3A forms a more stable dimer than Cry3Bb and coincidentally forms higher level conductances faster (FIG. 10). This observation led the inventors to propose that oligomerization and ion channel formation (conductance size and speed of channel formation) were related. Based on this observation Cry3Bb was re-engineered to make larger and more stable oligomers at a faster rate. It is assumed in this analysis that the rate of ion channel formation and growth mirrors this process. It is also possible that changes in quaternary structure may not affect channel activity alone or at all. Alterations to quaternary structure may also affect receptor interactions, protein processing in the insect gut environment, as well as other aspects of bioactivity unknown.

5.14.1 Cry3Bb.11048

Comparative structural analysis of Cry3A and Cry3Bb led to the identification of structural differences between the two toxins in the ion channel-forming domain, specifically, an insertion of one amino acid between helix 2a and helix 2b in Cry3Bb. Removal of this additional amino acid in Cry 3B2, A104, and a D103E substitution, as in Cry3A, resulted in loss of channel gating and the formation of symmetrical pores. Once the pores are formed they remain open and allow a steady conductance ranging from 25–139 pS. This designed protein, Cry3Bb.11048, is 4.3 times more active than WT Cry3Bb against CCRW larvae.

5.14.2 Oligomerization of Cry3Bb.60

Individual molecules of Cry3Bb or Cry3Bb.60 can form a complex with another like molecule. Oligomerization of Cry3Bb is demonstrated by SDS-PAGE, where sample are not heated in sample buffer prior to loading on the gel. The lack of heat treatment allows some nondenatured toxin to remain. Oligomerization is visualized following Coomassie staining by the appearance of a band at 2 times the molecular weight of the monomer. The intensity of the higher molecular weight band reflects the degree of oligomerization. The ability of Cry3Bb to form an oligomer is not reproducibly apparent. The complex cannot be repeatedly observed to form. Cry3Bb.60, however, forms a significantly greater amount of a higher molecular weight complex (120 kDa). These data suggest that Cry3Bb.60 more readily forms the higher order complex than Cry3Bb alone. Cry3Bb.60 also forms ion channels with greater frequency than WT Cry3Bb (see Section 5.12.9).

5.14.3 Cry3Bb.11035

Changes were made in Cry3Bb to reflect the amino acid sequence in Cry3A at the end of the α 3,4 and in the beginning of helix 4. These changes resulted in the designed protein, Cry3Bb.11035, that, unlike wild type Cry3Bb, forms spontaneous channels with large conductances. Cry3Bb.11035 is also approximately three times more active against SCRW larvae than WT Cry3Bb. Cry3Bb.11035 and its amino acid changes are listed in Table 10.

5.14.4 Cry3Bb.11032

Cry3Bb.11032 was altered at residue 165 in helix α 4, changing an aspartate to glycine, as found in Cry3A.

Cry3Bb.11032 is three-fold more active than WT Cry3Bb. The channel activity of Cry3Bb.11032 is much like Cry3Bb except when the designed protein is artificially incorporated into the membrane. A 16-fold increase in the initial channel conductances is observed compared to WT Cry3Bb (see Section 5.12.2). This increase in initial conductance presumably is due to enhanced quaternary structure, stability of higher-order structure.

5.14.5 EG11224

In the WT Cry3Bb dimer structure, histidine, at position 231 in domain 1, makes hydrogen bond contacts with D288 (domain 1), Y230 (domain 1), and, through a network of water molecules, also makes contacts to D610 (domain 3), all of the opposite monomer. D610 and K235 (domain 1) also make contact. Replacing the histidine with an arginine, H231R, results, in one orientation, in the formation of a salt bridge to D610 of the neighboring monomer. In a second orientation, the contacts with D288 of the neighboring monomers, as appear in the WT structure, are retained. In either orientation, I231 does not hydrogen bond to Y230 of the opposite monomer but does make contact with K235 which retains its contacts to K610 (V. Cody, research communication). The shifting hydrogen bonds have changed the interactions between the different domains of the protein in the quaternary structure. Overall, fewer hydrogen bonds exist between domains 1 of the neighboring monomers and a much stronger bond has been formed between domains 1 and 3. Channel activity was found to be altered. Cry3Bb.11224 produces small, quickly gating channels like Cry3Bb. However, unlike WT Cry3Bb, Cry3Bb.11224 does not exhibit, β -mercaptoethanol-dependent activation. Replacing H231 with arginine resulted in a designed Cry3Bb protein, Cry3Bb.11224, exhibiting a 5-fold increase in bioactivity.

5.14.6 Cry3Bb.11226

Cry3Bb.11226 is similar to Cry3Bb.11224, discussed in Section 4.8.5, in that the histidine at position 231 has been replaced. The amino acid change, H231T, results in the loss of β -mercaptoethanol dependent activation seen with WT Cry3Bb (see Section 5.12.1). The replacement of H231, a putative metal binding site, changes the interaction of regions in the quaternary structure resulting in a different type of channel activity. Cry3Bb.11226 is three-fold more active than WT Cry3Bb.

5.14.7 Cry3Bb.11221

Cry3Bb.11221 has been re-designed in the α 3,4 region of Cry3Bb. The channels formed by Cry3Bb.11221 are much more well resolved than the conductances formed by WT Cry3Bb (see Section 5.12.6). Cry3Bb.11221 exhibits a 6.4-fold increase in bioactivity over that of WT Cry3Bb. The amino acid changes found in Cry3Bb.11221 are listed in Table 2.

5.14.8 Cry3Bb.11242

The designed protein, Cry3Bb.11242, carrying the alteration R290V, forms small conductances immediately which grow rapidly and steadily to large conductances in about 3 min (see Section 5.12.7). This is contrast to WT Cry3Bb channels which take 30–45 min to appear and grow slowly over hours to large conductances. Cry3Bb.11242 also exhibits a 2.5-fold increase in bioactivity compared to WT Cry3Bb.

5.14.9 Cry3Bb.11230

Cry3Bb.11230, unlike WT Cry3Bb, forms well resolved channels with long open states. These channels reach a maximum conductance of 3000 pS but do not continue to grow with time. Cry3Bb.11230 has been re-designed in the 1 β , α 8 region of Cry3Bb and exhibits almost a 5-fold increase in activity against SCRW larvae (Table 9) and a 5.4-fold increase against WCRW larvae (Table 10) compared to WT Cry3Bb. The amino acid changes found in Cry3Bb.11230 are listed in Table 2.

5.15 Example 15

Design Method 9: Design of Structural Residues

The specific three-dimensional structure of a protein is held in place by amino acids that may be buried or otherwise removed from the surface of the protein. These structural determinants can be identified by inspection of forces responsible for the surface structure positioning. The impact of these structural residues can then be enhanced to restrict molecular motion or diminished to enhance molecular flexibility.

5.15.1 Cry3Bb.11095

Loops β 2,3, β 6,7 and β 10,11, located in domain 2 of Cry3Bb, have been identified as important for bioactivity. The three loops are surface-exposed and structurally close together. Amino acid Q348 in the WT structure, located in β -strand 2 just prior to 1 β 2,3, does not form any intramolecular contacts. However, replacing Q348 with arginine (Q348R) results in the formation of 2 new hydrogen-bonds between R348 and the backbone carbonyls of R487 and R488, both located in 1 β 10,11. The new hydrogen bonds may act to stabilize the structure formed by the three loops. Certainly, the structure around R348 is more lightly packed as determined by X-ray crystallography. The designed protein carrying this change, Cry3Bb.11095, is 4.6-fold more active than WT Cry3Bb.

5.16 Example 16

Design Method 10: Combinatorial Analysis and Mutagenesis

Individual sites in the engineered Cry3Bb molecule can be used together to create a Cry3Bb molecule with activity even greater than the activity of any one site. This method has not been precisely applied to any δ -endotoxin. It is also not obvious that improvements in two sites can be pulled together to improve the biological activity of the protein. In fact, data demonstrates that improvements to 2 sites, when pulled together into a single construct, do not necessarily further improve the biological activity of Cry3Bb. In some cases, the combination resulted in decreased protein stability and/or activity. Examples of proteins with site combinations that resulted in improved activity compared to WT Cry3Bb but decreased activity compared to 1 or more of the "parental" proteins are Cry3Bb.11235, 11046, 11057 and 11058. Cry3Bb.11082, which contains designed regions from 4 parental proteins, retains the level of activity from the most active parental strain (Cry3Bb.11230) but does not show an increase in activity. These proteins are listed in Table 7. The following are examples of instances where combined mutations having significantly improved biological activity.

5.16.1 Cry3Bb.11231

Designed protein Cry3Bb.11231 contains the alterations found in Cry3Bb.11224 (H231R) and Cry3Bb.11228

(changes in 1 β 1, α 8). The combination of amino acid changes found in Cry3Bb.11231 results in an increase in bioactivity against SCRW larvae of approximately 8-fold over that of WT Cry3B (Table 2). This increase is greater than exhibited by either Cry3Bb.11224 (5.0 $\times$) or Cry3Bb.11228 (4.1 $\times$) alone. Cry3Bb.11231 was also exhibits an 12.9-fold increase in activity compared to WT Cry3Bb against WCRW larvae (Table 10).

5.16.2 Cry3Bb.11081

Designed Cry3Bb protein Cry3Bb.11081 was constructed by combining the changes found in Cry3Bb.11032 and Cry3Bb.11229 (with the exception of Y318C). Cry3Bb.11081 a 6.1-fold increase in activity over WT Cry3Bb; a greater increase in activity than either of the individual parental proteins, Cry3Bb.11032 (3.1-fold) and Cry3Bb.11229 (2.5-fold).

5.16.3 Cry3Bb.11083

Designed Cry3Bb protein Cry3Bb.11083 was constructed by combining the changes found in Cry3Bb.11036 and Cry3Bb.11095. Cry3Bb.11083 exhibits a 7.4-fold increase in activity against SCRW larvae compared to W Cry3Bb; a greater increase than either Cry3Bb.11036 (4.3 $\times$) or Cry3Bb.11095 (4.6 $\times$). Cry3Bb.11083 also exhibits a 5.4-fold increase in activity against WCRW larvae compared to WT Cry3Bb (Table 10).

5.16.4 Cry3Bb.11084

Designed Cry3Bb protein Cry3Bb.11084 was constructed by combining the changes found in Cry3Bb.11032 and the S311L change found in Cry3Bb.11228. Cry3Bb.11084 exhibits a 7.2-fold increase in activity over that of WT Cry3Bb; a greater than either Cry3Bb.11032 (3.1 $\times$) or Cry3Bb.11228 (4.1 $\times$).

5.16.5 Cry3Bb.11098

Designed Cry3Bb protein Cry3Bb.11098 was constructed to contain the following amino acid changes: D165G, H231R, S311L, N313T, and E317K. The nucleic acid sequence is given in SEQ ID NO:107, and the encoded amino acid sequence is given in SEQ ID NO:108.

5.17 Example 17

Design Strategy 11: Alteration of Binding to Glycoproteins and to WCRW Brush Border Membranes

While the identity of receptor(s) for Cry3Bb is unknown, it is nonetheless important to increase the interaction of the toxin with its receptor. One way to improve the toxin-receptor interaction with knowing the identity of the receptor is to reduce or eliminate non-productive binding to other biomolecules. The inventors have observed that Cry3Bb binds non-specifically to bovine serum albumin (BSA) that has been glycosylated with a variety of sugar groups, but not to non-glycosylated BSA. Cry3A, which is not active on Diabrotica species, shows similar but even greater binding to glycosylated-BSA. Similarly, Cry3A shows greater binding to immobilized WCRW brush border membrane (BBM) than does WT Cry3Bb, suggesting that much of the observed binding is non-productive. It was reasoned that the non-specific binding to WCRW BBM occurs via glycosylated proteins, and that binding to both glycosylated-BSA and WCRW BBM is non-productive in reaction pathway to

toxicity. Therefore reduction or elimination of that binding would lead to enhanced binding to the productive receptor and to enhanced toxicity. Potential binding sites for sugar groups were targeted for redesign to reduce the non-specific binding of Cry3Bb to glycoproteins and to immobilized WCRW BBM.

5.17.1 Cry3Bb.60

Cry3Bb-60, in which Cry3Bb has been cleaved at R159 in 1 α 3,4 shows decreased binding to glycosylated-BSA and decreased binding to immobilized WCRW BBM. Cry3Bb60 shows a 3.6-fold increase in bioactivity relative to WT Cry3Bb.

5.17.2 Alterations to 1 α 3,4 (Cry3Bb.11221)

Cry3Bb.11221 has been redesigned in the 1 α 3,4 region of domain 1, which is the region in which Cry3Bb is cleaved to produce Cry3Bb-60. Cry3Bb.11221 also shows decreased binding to both glycosylated-BSA and immobilized WCRW BBM, and exhibits a 6.4-fold increase in bioactivity over that of WT Cry3Bb. Together with data for Cry3Bb.60 (section 5.17.1) these data suggest that this loop region contributes substantially to non-productive binding of the toxin.

5.17.3 Alteration to 1 β 1, α 8 (Cry3Bb.11228,11230, 11237 and 11231)

The 1 β 1, α 8 region of Cry3Bb has been re-engineered to increase hydration (section 4.2.4) and enhance flexibility (section 4.4.3). Several proteins altered in this region, Cry3Bb.11228,11230, and 11237 demonstrate substantially lower levels of binding both glycosylated-BSA and immobilized WCRW BBM, and also show between 4.1- and 4.5-fold increases in bioactivity relative to WT Cry3Bb.

5.17.4 Binding Activity

The tendencies of Cry3Bb and some of its derivatives to bind to glycosylated-BSA and to WCRW BBM were determined using a BIAcore™ surface plasmon resonance biosensor. For glycosylated-BSA binding, the glycosylated protein was immobilized using standard NHS chemistry to a CM5 chip (BIAcore), and the solubilized toxin was injected over the glycosylated-BSA surface. To measure binding to WCRW BBM, brush border membrane vesicles (BBMV) purified from WCRW midguts (English et al., 1991) were immobilized on an HPA chip (BIAcore) then washed with either 10 mM KOH or with 40 mM β -octylglucoside. The solubilized toxin was then injected over the resulting hybrid bilayer surface to detect binding. Protein concentration were determined by Protein Dye Reagent assay (BioRad) or BCA Protein Assay (Pierce). Other methods may also be used to determine the same binding information. These include, but are not limited to, ligand blot experiments using labeled toxin, labeled glycosylated protein, or anti-toxin antibodies, affinity chromatography, and in vitro binding of toxin to intact BBMV.

5.18 Example 18

Construction of Plasmids with WT Cry3Bb Sequences

Standard recombinant DNA procedures were performed essentially as described by Sambrook et al., (1989).

5.18.1 pEG1701

pEG1701 (FIG. 11), contained in EG11204 and EG11037, was constructed by inserting the SphI-PstI fragment con-

taining the cry3Bb gene and the cry1F terminator from pEG911 (Baum, 1994) into the SphI-PstI site of pEG854.9 (Baum et al., 1996), a high copy number B. thuringiensis-E. coli shuttle vector.

5.18.2 pEG1028

pEG1028 contains the HindIII fragment of cry3Bb from pEG1701 cloned into the multiple cloning site of pTZ18U at HindIII.

5.19 Example 19

Construction of Plasmids with Altered Cry3Bb Genes

Plasmid DNA from E. coli was prepared by the alkaline lysis method (Maniatis et al., 1982) or by commercial plasmid preparation kits (examples: PERFECTprep™ kit, 5 Prime-3 Prime, Inc., Boulder CO; QIAGEN plasmid prep kit, QIAGEN Inc.). B. thuringiensis plasmids were prepared from cultures grown in brain heart infusion plus 0.5% glycerol (BHIG) to mid logarithmic phase by the alkaline lysis method. When necessary for purification, DNA fragments were excised from an agarose gel following electrophoresis and recovered by glass milk using a GeneClean II® kit (BIO 101 Inc., La Jolla, Calif.). Alteration of the cry3Bb gene was accomplished using several techniques including site-directed mutagenesis, triplex PCR™, quasi-random PCR™ mutagenesis, DNA shuffling and standard recombinant techniques. These techniques are described in Sections 6.1, 6.2, 6.3, 6.4 and 6.5, respectively. The DNA sequences of primers used are listed in Section 7.

5.20 Example 20

Site-directed Mutagenesis

Site-directed mutagenesis was conducted by the protocols established by Kunkle (1985) and Kunkle et al. (1987) using the Muta-Gene™ M13 in vitro mutagenesis kit (Bio-Rad, Richmond, Calif.). Combinations of alterations to cry3Bb were accomplished by using the Muta-Gene™ kit and multiple mutagenic oligonucleotide primers.

5.20.1 pEG1041

pEG1041, contained in EG11032, was constructed using the Muta-Gene™ kit, primer C, and single-stranded pEG1028 as the DNA template. The resulting altered cry3Bb DNA sequence was excised as a PflMI DNA fragment and used to replace the corresponding DNA fragment in pEG1701.

5.20.2 pEG1046

pEG1046, contained in EG11035, was constructed using the Muta-Gene™ kit, primer D, and single-stranded pEG1028 as the DNA template. The resulting altered cry3Bb DNA sequence was excised as a PflMI DNA fragment and used to replace the corresponding DNA fragment in pEG1701.

5.20.3 pEG1047

pEG1047, contained in EG11036, was constructed using the Muta-Gene™ kit, primer E, and single-stranded pEG1028 as the DNA template. The resulting altered cry3Bb DNA sequence was excised as a PflMI DNA fragment and used to replace the corresponding DNA fragment in pEG1701.

5.20.4 pEG1052

pEG1052, contained in EG11046, was constructed using the Muta-Gene™ kit, primers D and E, and single-stranded pEG1028 as the DNA template. The resulting altered cry3Bb DNA sequence was excised as a PflMI DNA fragment and used to replace the corresponding DNA fragment in pEG1701.

5.20.5 pEG1054

pEG1054, contained in EG11048, was constructed using the Muta-Gene™ kit, primer F, and single-stranded pEG1028 as the DNA template. The resulting altered cry3Bb DNA sequence was excised as a PflMI DNA fragment and used to replace the corresponding DNA fragment in pEG1701.

5.20.6 pEG1057

pEG1057, contained in EG11051, was constructed using the Muta-Gene™ kit, primer G, and single-stranded pEG1028 as the DNA template. The resulting altered cry3Bb DNA sequence was excised as a PflMI DNA fragment and used to replace the corresponding DNA fragment in pEG1701.

5.21 Example 21

Triplex PCR™

Triplex PCR™ is described by Michael (1994). This method makes use of a thermostable ligase to incorporate a phosphorylated mutagenic primer into an amplified DNA fragment during PCR™. PCR™ was performed on a Perkin Elmer Cetus DNA Thermal Cycler (Perkin-Elmer, Norwalk, Conn.) using a AmpliTaq™ DNA polymerase kit (Perkin-Elmer) and SphI-linearized pEG1701 as the template DNA. PCR™ products were cleaned using commercial kits such as Wizard™ PCR™ Preps (Promega, Madison, Wis.) and QIAquick PCR™ Purification kit (QIAGEN Inc., Chatsworth, Calif.).

5.21.1 pEG1708 and pEG1709

pEG1708 and pEG1709, contained in EG11222 and EG11223, respectively, were constructed by replacing the PflMI-PflMI fragment of cry3Bb in pEG1701 with PflMI-digested and gel purified PCR™ fragment altered at cry3Bb nucleotide positions 688–690, encoding amino acid Y230. Random mutations were introduced into the Y230 codon by triplex PCR™. Mutagenic primer MVT095 was phosphorylated and used together with outside primer pair FW001 and FW006. Primer MVT095 also contains a silent mutation at position 687, changing T to C, which, upon incorporation, introduces an additional EcoRI site into pEG1701.

5.21.2 pEG1710, pEG1711 and pEG1712

Plasmids pEG1701, pEG1711 and pEG1712, contained in EG11224, EG11225 and EG11226, respectively, were created by replacing the PflMI-PflMI fragment of the cry3Bb gene in pEG1701 with PflMI-digested and gel purified PCR™ fragment altered at cry3Bb nucleotide positions 690–692, encoding H231. Random mutations were introduced into the H231 codon by triplex PCR™. Mutagenic primer MVT097 was phosphorylated and used together with outside primer pair FW001 and FW006. Primer MVT097 also contains a T to C sequence change at position 687 which, upon incorporation, results in an additional EcoRI site by silent mutation.

5.21.3 pEG1713 and pEG1727

pEG1713 and pEG1727, contained in EG11227 and EG11242, respectively, were constructed by replacing the PflMI-PflMI fragment of the cry3Bb gene in pEG1701 with PflMI-digested and gel purified PCR™ fragment altered at cry3Bb nucleotide positions 868–870, encoding amino acid R290. Triplex PCR™ was used to introduce random changes into the R290 codon. The mutagenic primer, MVT091, was designed so that the nucleotide substitutions would result in approximately 36% of the sequences encoding amino acids D or E. MVT091 was phosphorylated and used together with outside primer pair FW001 and FW006.

5.22 Example 22

Quasi-random PCR™ Mutagenesis

Quasi-random mutagenesis combines the mutagenic PCR™ techniques described by Vallette et al. (1989), Tomic et al. (1990) and LaBean and Kauffman (1993). Mutagenic primers, sometimes over 70 nucleotides in length, were designed to introduce changes over nucleotide positions encoding for an entire structural region, such as a loop. Degenerate codons typically consisted of a ratio of 82% WT nucleotide plus 6% each of the other 3 nucleotides per position to semi-randomly introduce changes over the target region (LaBean and Kauffman, 1993). When possible, natural restriction sites were utilized; class 2s enzymes were used when natural sites were not convenient (Stemmer and Morris, 1992, list additional restriction enzymes useful to this technique). PCR™ was performed on a Perkin Elmer Cetus DNA Thermal Cycler (Perkin-Elmer, Norwalk, Conn.) using a AmpliTaq™ DNA polymerase kit (Perkin-Elmer) and SphI-linearized pEG1701 as the template DNA. Quasi-random PCR™ amplification was performed using the following conditions: denaturation at 94° C. for 1.5 min.; annealing at 50° C. for 2 min. and extension at 72° C. for 3 min., for 30 cycles. The final 14 extension cycles were extended an additional 25 s per cycle. Primers concentration was 20 μM per reaction or 40 μM for long, mutagenic primers. PCR™ products were cleaned using commercial kits such as Wizard™ PCR™ Preps (Promega, Madison, Wis.) and QIAquick PCR™ Purification kit (QIAGEN Inc., Chatsworth, Calif.). In some instances PCR™ products were treated with Klenow Fragment (Promega) following the manufacturer's instructions to fill in any single base overhangs prior to restriction digestion.

5.22.1 pEG1707

EG1707, contained in EG11221, was constructed by replacing the PflMI-PflMI fragment of the cry3Bb gene in pEG1701 with PflMI-digested and gel purified PCR™ fragment altered at cry3Bb nucleotide positions 460–480, encoding 1α3,4 amino acids 154–160. Primer MVT075, which includes a recognition site for the class 2s restriction enzyme BsaI, and primer FW006 were used to introduce changes into this region by quasi-random mutagenesis. Primers MVT076, also containing a BsaI site, and primer FW001 were used to PCR™ amplify a "linker" fragment. Following PCR™ amplification, both products were cleaned, end-filled, digested with BsaI and ligated to each other. Ligated fragment was gel purified and used as template for PCR™ amplification using primer pair FW001 and FW006. PCR™ product was cleaned, digested with PflMI, gel purified and ligated into PflMI-digested and purified pEG1701 vector DNA.

5.22.2 pEG1720 and pEG1726

pEG1720 and pEG1726, contained in EG11234 and EG11241, respectively, were constructed by replacing the

PfIMI-PfIMI fragment of the cry3Bb gene in pEG1701 with PfIMI-digested and gel purified PCRTM fragment altered at cry3Bb nucleotide positions 859–885, encoding 1 α 7, β 1 amino acids 287–295. Quasi-random PCRTM mutagenesis was used to introduce changes into this region. Mutagenic primer MVT111, designed with a BsaI site, and primer FW006 were used to introduce the changes. Primer pair MVT094, also containing a BsaI site, and FW001 were used to amplify the linker fragment. The PCRTM products were digested with BsaI, gel purified then ligated to each other. Ligated product was PCRTM amplified using primer pair FW001 and FW006, digested with PfIMI.

5.22.3 pEG1714, pEG1715, pEG1716, pEG1718, pEG1719, pEG1722, pEG1723, pEG1724 and pEG1725

pEG1714, pEG1715, pEG1716, pEG1718, pEG1719, pEG1722, pEG1723, pEG1724 and pEG1725, contained in EG11228, EG11229, EG11230, EG11232, EG11233, EG11236, EG11237, EG11238 and EG11239, respectively, were constructed by replacing the PfIMI-PfIMI fragment of the cry3Bb gene in pEG1701 with PfIMI-digested and gel purified PCRTM fragment altered at cry3Bb nucleotide positions 931–954, encoding 1 β 1, α 8 amino acids 311–318. Quasi-random PCRTM mutagenesis was used to introduce changes into this region using mutagenic primer MVT103 and primer FW006. Primers FW001 and FW006 were used to amplify a linker fragment. The PCRTM products were end-filled using Klenow and digested with BamHI. The larger fragment from the FW001–FW006 digest was gel purified then ligated to the digested MVT103–FW006 fragment. Ligated product was gel purified and amplified by PCRTM using primer pair FW001 and FW006. The amplified product was digested with PfIMI and gel purified prior to ligation into PfIMI-digested and purified pEG1701 vector DNA.

5.22.4 pEG1701.1 β 2.3

Plasmids carrying alterations of cry3Bb WT sequence at nucleotides 1051–1065, encoding structural region 1 β 2,3 of Cry3Bb, were constructed by replacing the MluI-SpeI fragment of pEG1701 with isolated MluI- and SpeI-digested PCRTM product. The PCRTM product was generated by quasi-random PCRTM mutagenesis where mutagenic primer MVT081 was paired with FW006. These plasmids as a group are designated pEG1701.1 β 2,3.

5.22.5 pEG1701.1 β 6,7

Plasmids containing mutations of the cry3Bb WT sequence at nucleotides 1234–1248, encoding structural region 1 β 6,7 of Cry3Bb, were constructed by replacing the MluI-SpeI fragment of pEG1701 with isolated MluI- and SpeI-digested PCRTM product. The PCRTM product was generated by quasi-random PCRTM mutagenesis where mutagenic primer MVT085 was paired with primer WD115. Primer pair MVT089 and WD112 were used to amplify a linker fragment. Both PCRTM products were digested with TaqI and ligated to each other. The ligation product was gel purified and PCRTM amplified using primer pair MVT089 and FW006. The amplified product was digested with MluI and SpeI and ligated into MluI and SpeI digested and purified pEG1701 vector DNA. These plasmids as a group are designated pEG1701.1 β 6,7.

5.22.6 pEG1701.110,11

Plasmids containing mutated cry3Bb sequences at nucleotides 1450–1467, encoding structural region 1 β 10,11 of

Cry3Bb, were constructed by replacing the SpeI-PstI fragment of pEG1701 with isolated SpeI- and PstI-digested PCRTM product. The PCRTM product was generated by quasi-random PCRTM mutagenesis where mutagenic primer MVT105 was paired with primer MVT070. Primer pair MVT092 and MVT083 were used to generate a linker fragment. (MVT083 is a mutagenic oligo designed for another region. The sequence changes introduced by MVT083 are removed following restriction digestion and do not impact the alteration of cry3Bb in the 1 β 10,11 region.) Both PCRTM products were digested with BsaI, ligated together, and the ligation product PCRTM amplified with primer pair MVT083 and MVT070. The resulting PCRTM product was digested with SpeI and PstI, and gel purified. These plasmids as a group are designated pEG1701.1 β 10,11.

5.23 Example 23

DNA Shuffling

DNA-shuffling, as described by Stemmer (1994), was used to combine individual alterations in the cry3Bb gene.

5.23.1 pEG1084, pEG1085, pEG1086 and pEG1087

pEG1084, pEG1085, pEG1086, and pEG1087, contained in EG11081, EG11082, EG11083, and EG11084, respectively, were recovered from DNA-shuffling. Briefly, PfIMI DNA fragments were generated using primer set A and B and each of the plasmids pEG1707, pEG1714, pEG1715, pEG1716, pEG1041, pEG1046, pEG1047, and pEG1054 as DNA templates. The resulting DNA fragments were pooled in equal-molar amounts and digested with DNaseI and 50–100 bp DNA fragments were recovered from an agarose gel by three successive freeze-thaw cycles: three min in a dry-ice ethanol bath followed by complete thawing at 50° C. The recovered DNA fragments were assembled by primerless-PCRTM and PCRTM-amplified using the primer set A and B as described by Stemmer (1994). The final PCRTM-amplified DNA fragments were cut with PfIMI and used to replace the corresponding cry3Bb PfIMI DNA fragment in pEG1701.

5.24 Example 24

Recombinant DNA Techniques

Standard recombinant DNA procedures were performed essentially as described by Sambrook et al. (1989).

5.24.1 pEG1717

pEG1717, contained in EG11231, was constructed by replacing the small BglII fragment of pEG1710 with the small BglII fragment from pEG1714.

5.24.2 pEG1721

pEG1721, contained in EG11235, was constructed by replacing the small BglII fragment of pEG1710 with the small BglII fragment from pEG1087.

5.24.3 pEG1063

pEG1062, contained in EG11057, was constructed by replacing the NcoI DNA fragment containing ori 43 from pEG1054 with the isolated NcoI DNA fragment containing ori 43 and the alterations in cry3Bb from pEG1046.

5.24.4 pEG1063

pEG1063, contained in EG11058, was constructed by replacing the NcoI DNA fragment containing ori 43 from

pEG1054 with the isolated NcoI DNA fragment containing ori 43 and the alterations in cry3Bb from pEG1707.

5.24.5 pEG1095

pEG1095, contained in EG11095, was constructed by replacing the MluI-SpeI DNA fragment in pEG1701 with the corresponding MluI-SpeI DNA fragment from pEG1086.

5.25 Example 25

Primers Utilized in Constructing Cry3Bb* Variants

Shown below are the primers used for site-directed mutagenesis, triplex PCR™ and quasi-random PCR™ to prepare the cry3Bb* variants as described above. Primers were obtained from Ransom Hill Bioscience, Inc. (Ramona, Calif.) and Integrated DNA Technologies, Inc. (Coralville, Iowa). The specific composition of the primers containing particular degeneracies at one or more residues is given in Section 5.30, Example 30.

5.25.1 Primer FW001 (SEQ ID NO:71)

5'-AGACAACTCTACAGTAAAAGATG-3'

5.25.2 Primer FW006 (SEQ ID NO:72)

5'-GGTAATTGGTCAATAGAAATC-3'

5.25.3 Primer MVT095 (SEQ ID NO:73)

5'-CAGAAGATGTTGCTGAATTCNNNCATAGAC AATTAAAAC-3'

5.25.4 Primer MVT097 (SEQ ID NO:74)

5'-GATGTTGCTGAATTCJATNNNAGACAATTAA AAC-3'

5.25.5 Primer MVT091 (SEQ ID NO:75)

5'-CCCATTTTATGATATTBDNTTATACTCAAAGG-3'

5.25.6 Primer MVT075 (SEQ ID NO:76)

5'-AGCTATGCTGGTCTCGGAAGAAAEFNFNFJNJF JFJNFJNJFJAAAAGAAGCCAAGATCGAAT-3'

5.25.7 Primer MVT076 (SEQ ID NO:77)

5'-GGTCACCTAGGTCTCTCTTCCAGGAATTTAAC GCAITTAAC-3'

5.25.8 Primer MVT111 (SEQ ID NO:78)

5'-AGCTATGCTGGTCTCCCAJTTJEHIEJEJJEIHKRR JEHEJJEENIIIGTTAAAACAGAACTAAC-3'

5.25.9 Primer MVT094 (SEQ ID NO:79)

5'-ATCCAGTGGGGTCTCAAATGGGAAAAGTACA ATTAG-3'

5.25.10 Primer MVT103 (SEQ ID NO:80)

5'-CATTTTTACGGATCCAATTTTTJFFFJNEEJEFNFJ NFEILEJEOGGACCAACTTTTTTGAG-3'

5.25.11 Primer MVT081 (SEQ ID NO:81)

5'-GAATTCATACGCGTCTTCAACCTGGTJEHJJJIN MEEIEJCTTTCAATTATTGGTCTGG-3'

5.25.12 Primer MVT085 (SEQ ID NO:82)

5'-AAAAGTTTATCGAACFATAGCTAATACAGACGT AGCGGCTJQQFFNEEJIIJEEIGTATATTTAGGTGT TACG-3'

5.25.13 Primer A (SEQ ID NO:83) 3b2pflm1

5'-GGAGTTCCATTGCTGGGGC-3'

5.25.14 Primer B (SEQ ID NO:84) 3b2pflm2

5'-ATCTCCATAAAATGGGG-3'

5.25.15 Primer C (SEQ ID NO:85) 3b2165DG

5'-GCGAAGTAAAAGAAGCCAAGGTCGAATAA GGG-3'

5.25.16 Primer D (SEQ ID NO:86) 3b2160SKRD

5'-CCTTTAAGTTTTCGCAAATCCACACAGCCAA GGTCGAATAAGGG-3'

5.25.17 Primer E (SEQ ID NO:87) 3b2290 VP

5'-CCCATTTTATGATGTTTCGGTTATACCCAAAA GGGG-3'

5.25.18 Primer F (SEQ ID NO:88) 3b2EdA104

5'-GGCCAAGTGAAGACCCATGGAAGGC-3'

5.25.19 Primer G (SEQ ID NO:89) 3b2KG189

5'-GCAGTTTCCGGATTCTGAAGTGC-3'

5.25.20 Primer WD112 (SEQ ID NO:90)

5'-CCGCTACGTCTGTATTA-3'

5.25.21 Primer WD115 (SEQ ID NO:91)

5'-ATAATGGAAGCACCTGA-3'

5.25.22 Primer MVT105 (SEQ ID NO:92)

5'-AGCTATGCTGGTCTCTTCTTAEJIFEIIEFFIJFIJIN ACAATTCCATTTTTTACTTGG-3'

5.25.23 Primer MVT092 (SEQ ID NO:93)

5'-ATCCAGTTGGGTCTCTAAGAAACAAACCGC GTAATTAAGC-3'

5.25.24 Primer MVT070 (SEQ ID NO:94)

5'-CCTCAAGGGTTATAACATCC-3'

5.25.25 Primer MVT083 (SEQ ID NO:95)

5'-GTACAAAAGCTAAGCTTTTIEJINPEEMEEIJN JESCGAAGTATAGCTAATACAG-3'

5.26 Example 26

Sequence Analysis of Altered Cry3Bb Genes

E. coli DH5α™ (GIBCO BRL, Gaithersburg, Md.), JM110 and Sure™ (Stratagene, La Jolla, Calif.) cells were sometimes used amplify plasmid DNA for sequencing. Plasmids were transformed into these cells using the manufacturers' procedures. DNA was sequenced using the Sequenase® 2.0 DNA sequencing kit purchased from U.S. Biochemical Corporation (Cleveland, Ohio). The plasmids described in Section 6, their respective divergence from WT cry3Bb sequence, the resulting amino acid changes and the protein structure site of the changes are listed in Table 11.

TABLE 11

DNA SEQUENCE CHANGES OF CRY3BB* GENES AND RESULTING AMINO ACID SUBSTITUTIONS OF THE CRY3BB* PROTEINS			
Plasmid	cry3Bb* DNA Sequence	Cry3Bb* Amino Acid Sequence	Structural Site of Alteration
pEG1707	A460T, C461T, A462T, C464A, T465C, T466C, T467A, A468T, A469T, G470C, T472C, T473G, G474T, A477T, A478T, G479C	T154F, P155H, L156H, L158R	1 α 3, 4
pEG1708	T687C, T688C, A689T, C691A, A692G	Y230L, H231S	α 6
pEG1709	T667C, T687C, T688A, A689G, C691A, A692G	S223P, Y230S	α 6
pEG1710	T687C, A692G	H231R	α 6
pEG1711	T687C, C691A	H231N, T241S	α 6
pEG1712	T687C, C691A, A692C, T693C	H231T	α 6
pEG1713	C868A, G869A, G870T	H290N	1 α 7, β 1
pEG1714	C932T, A938C, T942G, G949A, T954C	S311L, N313T, E317K	1 β 1, α 8
pEG1715	T931A, A933C, T942A, T945A, G949A, A953G, T954C	S311T, E317K, Y318C	1 β 1, α 8
pEG1716	T931G, A933C, C934G, T945G, C946T, A947G, G951A, T954C	S311A, L312V, Q316W	1 β 1, α 8
pEG1717	T687C, A692G, C932T, A938C, T942G, G949A, T954C	H231R, S311L, N313T, E317K	α 6, 1 β 1, α 8
pEG1718	T931A, A933G, T935C, T936A, A938C, T939C, T942C, T945A, G951T, T954C	S311T, L312P, N313T, B317N	1 β 1, α 8
pEG1719	T931G, A933C, T936G, T942C, C943T, T945A, C946G, G948C, T954C	S311A, Q316D	1 β 1, α 8
pEG1720	T861C, T866C, C868A, T871C, T872G, A875T, T877A, C878G, A882G	I289T, L291R, Y292F, S293R	1 α 7, β 1
pEG1721	T687C, A692G, C932T	H231R, S311L	α 6, 1 β 1, α 8
pEG1722	T931A, C932T, A933C, T936C, T942G, T945A, T954C	S311I	1 β 1, α 8
pEG1723	T931A, C932T, A933C, T936C, A937G, A938T, C941A, T942C, T945A, C946A, A947T, A950T, T954C	S311I, N313H	1 β 1, α 8
pEG1724	A933C, T936C, A937G, A938T, C941A, T942C, T945A, C946A, A947T, A950T, T954C	N313V, T314N, Q316M, E317V	1 β 1, α 8
pEG1725	A933T, A938G, T939G, T942A, T944C, T945A, A947T, G948T, A950C, T954C	N313R, L315P, Q316L, E317A	1 β 1, α 8
pEG1726	A860T, T861C, G862A, C868T, G869T, T871C, A873T, T877A, C878G, A879T	Y287F, D288N, R290L	1 α 7, β 1
pEG1727	C868G, G869T	R290V	1 α 7, β 1
pE41041	A494G	D165G	α 4
pEG1046	G479A, A481C, A482C, A484C, G485A, A486C, A494G	S160N, K161P, [P162H] <i>R162H</i> , D165G	α 4
pEG1047	A865G, T877C	I289V, S293P	1 α 7, β 1
pEG1052	G479A, A481C, A482C, A484C, G485A, A486C, A494G, A865G, D165G, T877C	S160N, K161P, [P162H] <i>R162H</i> , D165G, I289V, S293P	α 4, 1 α 7, β 1
pEG1054	T309A, Δ 310, Δ 311, Δ 312	D103E, Δ A104	1 α 2a, 2b
pEG1057	A565G, A566G	K189G	1 α 4, 5
pEG1062	T309A, Δ 310, Δ 311, Δ 312, G479A, A481C, A482C, A484C, G485A, A486C, A494G	D103E, Δ A104, S160N, K161P, [P162H] <i>R162H</i> , <i>D165G</i>	1 α 2a, 2b α 4
pEG1063	T309A, Δ 310, Δ 311, Δ 312, A460T, C461T, A462T, C464A, T465C, T466C, T467A, A468T, A469T, G470C, T472C, T473G, G474T, A477T, A478T, G479C	D103E, Δ A104, T154F, P155H, L156H, L158R	1 α 2, 2b 1 α 3, 4
pEG1084	A494G, T931A, A933C, T942A, T945A, G949A, T954C	D165G, S311T, E317K	α 4, 1 β 1, α 8
pEG1085	A494G, A865G, T877C, T914C, T931G, A933C, C934G, T945G, C946T, A947G, G951A, T954C, A1043G, T1094C	D165G, I289V, S293P, F305S, S311A, L312V, Q316W, Q348R, V365A	α 4, 1 α 7, β 1 β 1, 1 β 1, α 8 β 2, β 3b
pEG1086	A865G, T877C, A1043G	I289V, S293P, Q348R	1 α 7, β 1, β 2
pEG1087	A494G, C932T	D165G, S311L	α 4, 1 β 1, α 8
pEG1095	A1043G	Q348R	β 2

5.27 Example 27

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Expression of Cry3Bb* Proteins

5.27.1 Culture Conditions

LB agar was prepared using a standard formula (Maniatis et al., 1982). Starch agar was obtained from Difco Laboratories (Detroit, Mich.) and supplemented with an additional 5 g/l of agar. C2 liquid medium is described by Donovan et al. (1988). C2 medium was sometimes prepared without the phosphate buffer (C2-P). All cultures were incubated at 25° C. to 30° C.; liquid cultures were also shaken at 250 rpm, until sporulation and lysis had occurred.

5.27.2 Transformation Conditions

pEG1701 and derivatives thereof were introduced into acrySTALLIFEROUS *B. thuringiensis* var. *kurstaki* EG7566 (Baum, 1994) or EG10368 (U.S. Pat. No. 5,322,687) by the electroporation method of Macaluso and Mettus (1991). In some cases, the method was modified as follows to maximize the number of transformants. The recipient *B. thuringiensis* strain was inoculated from overnight growth at 30° C., on LB agar into brain heart infusion plus 0.5% glycerol, grown to an optical density of approximately 0.5 at 600 nm, chilled on ice for 10 min, washed 2 $\times$ with EB and resuspended in a 1/50 volume of EB. Transformed cells were selected on LB agar or starch agar plus 5 μ g/ml chloram-

phenicol. Visual screening of colonies was used to identify transformants producing crystalline protein; those colonies were generally more opaque than colonies that did not produce crystalline protein.

5.27.3 Strain and Protein Designations

A transformant containing an altered cry3Bb* gene encoding an altered Cry3Bb* protein is designated by an "EG" number, e.g., EG11231. The altered Cry3Bb* protein is designated Cry3Bb followed by the strain number, e.g., Cry3Bb.11231. Collections of proteins with alterations at a structural site are designated Cry3Bb followed by the structural site, e.g., Cry3Bb.1β2,3. Table 12 lists the plasmids pertinent to this invention, the new *B. thuringiensis* strains containing the plasmids, the acrySTALLIFEROUS *B. thuringiensis* recipient strain used, and the proteins produced by the new strains.

5.28 Example 28

Generation and Characterization of Cry3Bb-60

5.28.1 Generation of Cry3Bb-60

Cry3Bb-producing strain EG7231 (U.S. Pat. No. 5,187,091) was grown in C2 medium plus 3 mg/ml chloramphenicol. Following sporulation and lysis, the culture was washed with water and Cry3Bb protein purified by the NaBr solubilization and recrystallization method of Cody et al. (1992). Protein concentration was determined by BCA Protein Assay (Pierce, Rockford, Ill.). Recrystallized protein was solubilized in 10 ml of 50 mM KOH per 100 mg of Cry3Bb protein and buffered to pH 9.0 with 100 mM CAPS (3-[cyclohexylamino]-1-propanesulfonic acid), pH 9.0. The soluble toxin was treated with trypsin at a weight ratio of 50 mg toxin to 1 mg trypsin for 20 min to overnight at room temperature. Trypsin cleaves proteins on the carboxyl side of available arginine and lysine residues. For 8-dose bioassay, the solubilization conditions were altered slightly to increase the concentration of protein: 50 mM KOH was added dropwise to 2.7 ml of a 12.77 mg/ml suspension of purified Cry3Bb* until crystal solubilization occurred. The volume was then adjusted to 7 ml with 100 mM CAPS, pH 9.0.

TABLE 12

PLASMIDS CARRYING ALTERED CRY3BB* GENES TRANSFORMED INTO <i>B. THURINGIENSIS</i> FOR EXPRESSION OF ALTERED CRY3BB* PROTEINS		
Plasmid Designation	New BT Strain	Expressed Protein
pEG1701	EG11204	WT Cry3Bb
pEG1701	EG11037	WT Cry3Bb
PEG1707	EG11221	Cry3Bb.11221
pEG1708	EG11222	Cry3Bb.11222
pEG1709	EG11223	Cry3Bb.11223
pEG1710	EG11224	Cry3Bb.11224
pEG1711	EG11225	Cry3Bb.11225
pEG1712	EG11226	Cry3Bb.11226
pEG1713	EG11227	Cry3Bb.11227
pEG1714	EG11228	Cry3Bb.11228
pEG1715	EG11229	Cry3Bb.11229
pEG1716	EG11230	Cry3Bb.11230
pEG1717	EG11231	Cry3Bb.11231
pEG1718	EG11232	Cry3Bb.11232
pEG1719	EG11233	Cry3Bb.11233
pEG1720	EG11234	Cry3Bb.11234
pEG1721	EG11235	Cry3Bb.11235
pEG1722	EG11236	Cry3Bb.11236

TABLE 12-continued

PLASMIDS CARRYING ALTERED CRY3BB* GENES TRANSFORMED INTO <i>B. THURINGIENSIS</i> FOR EXPRESSION OF ALTERED CRY3BB* PROTEINS		
Plasmid Designation	New BT Strain	Expressed Protein
pEG1723	EG11237	Cry3Bb.11237
pEG1724	EG11238	Cry3Bb.11238
pEG1725	EG11239	Cry3Bb.11239
pEG1726	EG11241	Cry3Bb.11241
pEG1727	EG11242	Cry3Bb.11242
pEG1041	EG11032	Cry3Bb.11012
pEG1046	EG11035	Cry3Bb.11035
pEG1047	EG11036	Cry3Bb.11036
pEG1052	EG11046	Cry3Bb.11046
pEG1054	EG11048	Cry3Bb.11048
pEG1057	EG11051	Cry3Bb.11051
pEG1062	EG11057	Cry3Bb.11057
pEG1063	EG11058	Cry3Bb.11058
pEG1084	EG11081	Cry3Bb.11081
pEG1085	EG11082	Cry3Bb.11082
pEG1086	EG11083	Cry3Bb.11083
pEG1087	EG11084	Cry3Bb.11084
pEG1095	EG11095	Cry3Bb.11095
pEG1098	EG11098	Cry3Bb.11098
pEG1701.1β2,3	collection of unnamed strains	Cry3Bb.1β2,3
pEG1701.1β6,7	collection of unnamed strains	Cry3Bb.1β6,7
pEG1701.1β10,11	collection of unnamed strains	Cry3Bb.1β10,11

5.28.2 Determination of Molecular Weight of Cry3Bb-60

The molecular weight of the predominant trypsin digestion fragment of Cry3Bb was determined to be 60 kDa by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis using commercial molecular weight markers. This digestion fragment is designated Cry3Bb-60. No further digestion of the 60 kDa cleavage product was observed.

5.28.3 Determination of NH₂-terminus of Cry3Bb-60

To determine the NH₂-terminal sequence of Cry3Bb-60, the trypsin digest was fractionated by SDS-PAGE and transferred to Immobilon™-P membrane (Millipore Corporation, Bedford, Mass.) following standard western blotting procedures. After transfer, the membrane was rinsed twice with water then stained with 0.025% Coomassie Brilliant Blue R-250 plus 40% methanol for 5 min, destained with 50% methanol and rinsed in water. The Cry3Bb.60 band was excised with a razor blade. NH₂-terminal sequencing was performed at the Tufts Medical School, Department of Physiology (Boston, Mass.) using standard automated Edman degradation procedures. The NH₂-terminal amino acid sequence was determined to be SKRSQDR (SEQ ID NO:96), corresponding to amino acids 160–166 of Cry3Bb. Trypsin digestion occurred on the carboxyl side of amino acid R159 resulting in the removal of helices 1–3.

5.29 Example 29

Bioactivity of Cry3Bb* Proteins

5.29.1 Culture Conditions and Protein Concentration Determination

Cultures for 1-dose bioassays were grown in C2-P plus 5 μg/ml chloramphenicol (C2-P/cm5) then diluted with 3 volumes of 0.005% Triton X-100®. The protein concentrations of these cultures were not determined. Cultures for 8-dose bioassays were grown in C2/cm5, washed 1–2 times

with 1–2 volumes of sterile water and resuspended in 1/10 volume of sterile 0.005% Triton X-100®. The toxin protein concentration of each concentrate was determined as described by Brussock and Currier (1990), omitting the treatment with 3 M HEPES. The protein concentration was adjusted to 3.2 mg/ml in 0.005% Triton X-100® for the top dose of the assay. Cry3Bb.60 was produced and quantified for 8-dose assay as described in Section 9.1.

5.29.2 Insect Bioassays

Diabrotica undecimpunctata howardi Barber (southern corn rootworm or SCRW) and *Diabrotica virgifera virgifera* LeConte (western corn rootworm or WCRW) larvae were reared as described by Slaney et al. (1992). Eight-dose assays and probit analyses were performed as described by Slaney et al. (1992). Thirty-two larvae were tested per dose at 50 µl of sample per well of diet (surface area of 175 mm²). Positive controls were WT Cry3Bb-producing strains EG11037 or EG11204. All bioassays were performed using 128-well trays containing approximately 1 ml of diet per well with perforated mylar sheet covers (C-D International Inc., Pitman, N.J.). One-dose assays were performed essentially the same except only 1 dose was tested per strain. All assay were replicated at least twice.

5.29.3 Insect Bioassay Results: 1-Dose Assays Against SCRW

Results from 1-dose assays are expressed as the relative mortality (RM) of the experimental strain compared to WT (% mortality of experimental culture divided by % mortality of WT culture). Altered and improved Cry3Bb proteins derived from plasmids constructed using PCR™ methods introducing random or semi-random changes into the cry3Bb gene sequence were distinguished from other altered but not improved Cry3Bb proteins by replicated, 1-dose assay against SCRW larvae. Those proteins showing increased activity (defined as RM ≥ 1.5) compared to WT Cry3Bb or, in the case of proteins with combinations of altered sites, compared to a “parental” altered Cry3Bb protein were further characterized by 8-dose assay. The overall RM “pattern” produced by 1-dose assay results from a collection of proteins carrying random or semi-random alterations within a single structural region, e.g., in 1β2,3, can be used to determine if that structural region is important for bioactivity. Retention of WT levels of activity (RM ≈ 1) indicate changes are tolerated in that region. Overall loss of activity (RM < 1) distinguishes the region as important for bioactivity.

5.29.4 Cry3Bb.1β2,3: Results of 1-Dose Bioassays Against SCRW

Cry3Bb.1β2,3 protein are a collection of proteins altered in the 1β2,3 region of Cry3Bb (see Section 5.3.4). Typical results of 1-dose assays of these altered proteins are shown in FIG. 12. The RM values for Cry3Bb.1β2,3 proteins are less than 1, with a few exceptions of values close to 1, indicating that this region is important for toxicity.

5.29.5 Cry3Bb.1β6,7: Results of 1-Dose Bioassays Against SCRW

Cry3Bb.1β6,7 proteins are a collection of proteins altered in the 1β6,7 region of Cry3Bb (see Section 5.3.5). Typical results of 1-dose assays of these altered proteins are shown in FIG. 13. With a few exceptions of values close to 1, the RM values for Cry3Bb.1β6,7 proteins are less than 1, indicating that this region is important for toxicity.

5.29.6 Cry3Bb.1β10,11: Results of 1-Dose Bioassays Against SCRW

Cry3Bb.1β10,11 proteins are a collection of proteins altered in the 1β10,11 region of Cry3Bb (see Section 5.3.6). Typical results of 1-dose assays of these altered proteins are shown in FIG. 14. With a few exceptions of values close to 1, the RM values for Cry3Bb.1β10,11 proteins are less than 1, indicating that this region is important for bioactivity.

5.29.7 Insect Bioassay Results: Results of 8-Dose Assays Against SCRW

Results from 8-dose assays are expressed as an LC₅₀ value (protein concentration giving 50% mortality) with 95% confidence intervals. The LC₅₀ values with 95% confidence intervals of altered Cry3Bb proteins showing improved activities against SCRW larvae and LC₅₀ values of the WT Cry3Bb control determined at the same time are listed in Table 13 along with the fold increase over WT activity for each improved protein.

TABLE 13

DESIGNED CRY3BB PROTEINS WERE TESTED AGAINST SCREW LARVAE IN REPLICATED, 8-DOSE ASSAYS TO DETERMINE THE IC₅₀ VALUES

Improved Protein	IC ₅₀ µg/well (95% C.I.)		Fold Increase Over WT Activity
	Improved Protein	WT Cry3Bb Control	
Cry3Bb.60	6.7 (5.3–8.4)	24.1 (15–39)	3.6x
Cry3Bb.11221	3.2 (2.5–4)	20.5 (14.5–29)	6.4x
Cry3Bb.11222	7.3 (6–9)	29.4 (23–37)	4.0x
Cry3Bb.11223	19.5 (9–12)	29.4 (23–37)	2.8x
Cry3Bb.11224	6.5 (5.1–8.2)	32.5 (25–43)	5.0x
Cry3Bb.11225	13.7 (11–16.8)	49.5 (39–65)	3.6x
Cry3Bb.11226	16.7 (10.6–24.2)	49.5 (39–65)	3.0x
Cry3Bb.11227	11.1 (9.1–13.5)	21.3 (16–28)	1.9x
Cry3Bb.11228	8.0 (6.6–9.8)	32.9 (25–45)	4.1x
Cry3Bb.11229	7.2 (5.8–8.8)	18.2 (15–22)	2.5x
Cry3Bb.11230	7.0 (5.8–8.6)	32.9 (25–45)	4.7x
Cry3Bb.11231	3.3 (3.0–3.7)	26.1 (22–31)	7.9x
Cry3Bb.11232	6.4 (5.4–7.7)	32.9 (25–45)	5.1x
Cry3Bb.11233	15.7 (12–20)	32.9 (25–45)	2.2x
Cry3Bb.11234	7 (6–9)	29 (22–39)	4.1x
Cry3Bb.11235	4.2 (3.6–4.9)	13.3 (10–17)	3.2x
Cry3Bb.11236	11.6 (9–15)	36.4 (27–49)	3.1x
Cry3Bb.11237	6.8 (4–11)	36.4 (27–49)	5.4x
Cry3Bb.11238	13.9 (11–17)	36.4 (27–49)	2.6x
Cry3Bb.11239	13.0 (10–16)	36.4 (27–49)	2.8x
Cry3Bb.11241	11 (7–16)	29 (22–39)	2.6x
Cry3Bb.11242	11.9 (9.2–16)	30 (23–38)	2.5x
Cry3Bb.11032	4.2 (3.6–4.9)	13.3 (10–17)	3.1x
Cry3Bb.11035	10.3 (8–13)	27.9 (23–34)	2.7x
Cry3Bb.11036	6.5 (5.1–7.9)	27.9 (23–34)	4.3x
Cry3Bb.11046	12.1 (8–19)	31.2 (25–39)	2.6x
Cry3Bb.11048	8.3 (6–11)	35.4 (24–53)	4.3x
Cry3Bb.11051	11.8 (8–16)	35.4 (24–53)	3.0x
Cry3Bb.11057	8.8 (7–11)	29.5 (24–36)	3.4x
Cry3Bb.11058	9.6 (6–14)	33.4 (27–43)	3.5x
Cry3Bb.11081	8.5 (7–11)	51.5 (37–79)	6.1x
Cry3Bb.11082	10.6 (8–13)	51.5 (37–79)	4.9x
Cry3Bb.11083	7.0 (5–10)	51.5 (37–79)	7.4x
Cry3Bb.11084	7.2 (4–12)	51.5 (37–79)	7.2x
Cry3Bb.11095	11.1 (9–14)	51.5 (37–79)	4.6x
Cry3Bb.11098			

5.29.8 Insect Bioassay Results: 8-Dose Assays Against WCRW

WCRW larvae are delicate and difficult to work with. Therefore, only some of the designed Cry3Bb showing improved activity against SCRW larvae were also tested against WCRW larvae in 8-dose assays. The LC₅₀ determi-

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nations for the designed Cry3Bb proteins are shown in Table 14 along with the LC₅₀ values of the WT Cry3Bb control determined at the same time.

TABLE 14

CRY3BB* PROTEINS SHOWING IMPROVED ACTIVITY AGAINST SCRW LARVAE ALSO SHOW IMPROVED ACTIVITY AGAINST WCRW LARVAE			
Improved Protein	IC ₅₀ µg/well (95% C.I.)		Fold Increase
	Improved Protein	WT Cry3Bb Control	Over WT Activity
EG11083	6.3 (4.7–8.2)	63.5 (46–91)	10.1x
EG11230	4.5 (2.1–7.4)	24.2 (13–40)	5.4x
EG11231	2.5 (1.7–3.6)	32.2 (14–67)	12.9x

5.30 Example 30

Channel Activity

Ion channels produced by Cry3Bb and some of its derivatives were measured by the methods described by Slatin et al. (1990). In some instances, lipid bilayers were prepared from a mixture of 4:1 phosphatidylethanolamine (PE): phosphatidylcholine (PC). Toxin protein was solubilized from washed, C2 medium, *B. thuringiensis* cultures with 12 mM KOH. Following centrifugation to remove spores and other debris, 10 µg of soluble toxin protein was added to the cis compartment (4.5 ml volume) of the membrane chamber. Protein concentration was determined using the BCA Protein Assay (Pierce).

5.30.1 Channel Activity of WT Cry3Bb.

Upon exposure to black lipid membranes, Cry3Bb forms ion channels with various conductance states. The channels formed by Cry3Bb are rarely discrete channels with well resolved open and closed states and usually require incubation of the toxin with the membrane for 30–45 min before any channel-like events are observed. After formation of the initial conductances, the size increases from approximately 200 pS to over 10,000 pS over 2–3 h. Only the small conductances (≤ 200 pS) are voltage dependent. Over 200 pS, the conductances are completely symmetric. Cry3Bb channels also exhibit β -mercaptoethanol-dependent activation, growing from small channel conductances of ~200 pS to several thousand pS within 2 min of the addition of β -mercaptoethanol to the cis compartment of the membrane chamber.

5.30.2 Cry3Bb.11032

The channel activity of Cry3Bb.11032 is much like WT Cry3Bb when the solubilized toxin protein is added to the cis compartment of the membrane chamber. However, when this protein is artificially incorporated into the membrane by forming or "painting" the membrane in the presence of the Cry3Bb.11032 protein, a 16-fold increase in the initial channel conductances is observed (~4000 pS). This phenomenon is not observed with WT Cry3Bb.

5.30.3 Cry3Bb.11035

Upon exposure to artificial membranes, the Cry3Bb.11035 protein spontaneously forms channels that grow to large conductances within a relatively short time span (~5 min). Conductance values ranges from 3000–6000 pS and, like WT Cry3Bb, are voltage dependent at low conductance values.

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5.30.4 Cry3Bb.11048

The Cry3Bb.11048 protein is quite different than WT Cry3Bb in that it appears not to form channels at all, but, rather, forms symmetrical pores with respect to voltage. Once the pore is formed, it remains open and allows a steady conductance ranging from 25 to 130 pS.

5.30.5 Cry3Bb.11224 and Cry3Bb.11226

The metal binding site of WT Cry3Bb formed by H231 in the dimer structure was removed in proteins Cry3Bb.11224 and Cry3Bb.11226. The conductances formed by both designed proteins are identical to that of WT Cry3Bb with the exception that neither of the designed proteins exhibits β -mercaptoethanol-dependent activation.

5.30.6 Cry3Bb.11221

Cry3Bb.11221 protein has been observed to immediately form small channels of 100–200 pS with limited voltage dependence. Some higher conductances were observed at the negative potential. In other studies, the onset of activity was delayed by 27 min, which is more typical for WT Cry3Bb. Unlike WT Cry3Bb, however, Cry3Bb.11221 forms well resolved, 600 pS channels with long open states. The protein eventually reaches conductances of 7000 pS.

5.30.7 Cry3Bb.11242

Cry3Bb.11242 protein forms small conductances immediately upon exposure to an artificial membrane. The conductances grow steadily and rapidly to 6000 pS in approximately 3 min. Some voltage dependence was noted with a preference for a negative imposed voltage.

5.30.8 Cry3Bb.11230

Unlike WT Cry3Bb, Cry3Bb.11230 forms well resolved channels with long open states that do not continue to grow in conductance with time. The maximum observed channel conductances reached 3000 pS. FIG. 15 illustrates the difference between the channels formed by Cry3Bb and Cry3Bb.11230.

5.30.9 Cry3Bb.60

Cry3Bb.60 forms well resolved ion channels within 20 min of exposure to an artificial membrane. These channels grow in conductance and frequency with time. The behavior of Cry3Bb.60 in a planar lipid bilayer differs from Cry3Bb in two significant ways. The conductances created by Cry3Bb.60 form more quickly than Cry3Bb and, unlike Cry3Bb, the conductances are stable, having well resolved open and closed states definitive of stable ion channels (FIG. 16).

TABLE 15

SEQ ID NO:83	% of Nucleotide in mixture			
	A	T	G	C
N	25	25	25	25

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TABLE 16

SEQ ID NO:84	% of Nucleotide in mixture			
Code	A	T	G	C
N	25	25	25	25

TABLE 17

SEQ ID NO:85	% of Nucleotide in mixture			
Code	A	T	G	C
B	16	16	52	16
D	70	10	10	10
N	25	25	25	25

TABLE 18

SEQ ID NO:86	% of Nucleotide in mixture			
Code	A	T	G	C
E	82	6	6	6
F	6	6	6	82
J	6	82	6	6
I	6	6	82	6
N	25	25	25	25

TABLE 19

SEQ ID NO:88	% of Nucleotide in mixture			
Code	A	T	G	C
J	6	82	6	6
E	82	6	6	6
H	1	1	1	97
I	6	6	82	6
K	15	15	15	55
R	15	55	15	15

TABLE 20

SEQ ID NO:90	% of Nucleotide in mixture			
Code	A	T	G	C
J	6	82	6	6
F	6	6	6	82
N	25	25	25	25
E	82	6	6	6
I	6	6	82	6
L	8	1	83	8
O	1	1	1	97

TABLE 21

SEQ ID NO:91	% of Nucleotide in mixture			
Code	A	T	G	C
J	6	82	6	6
E	82	6	6	6
H	1	1	1	97
I	6	6	82	6
N	25	25	25	25
M	82	2	8	8

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TABLE 22

SEQ ID NO:92		% of Nucleotide in mixture			
5	Code	A	T	G	C
10	J	6	82	6	6
	Q	0	9	82	9
	F	6	6	6	82
	N	25	25	25	25
	E	82	6	6	6
	I	6	6	82	6

TABLE 23

15	SEQ ID NO:92	% of Nucleotide in mixture			
	Code	A	T	G	C
20	J	6	82	6	6
	F	6	6	6	82
	N	25	25	25	25
	E	82	6	6	6
	I	6	6	82	6

TABLE 24

SEQ ID NO:95		% of Nucleotide in mixture			
	Code	A	T	G	C
30	J	6	82	6	6
	N	25	25	25	25
	E	82	6	6	6
	I	6	6	82	6
	M	82	2	8	8
	P	8	2	8	82
35	S	1	97	1	1

5.32 Example 32

Atomic Coordinates for Cry3Bb

The atomic coordinates of the Cry3Bb protein are given in the Appendix included in Section 9.1

5.33 Example 33

Atomic Coordinates for Cry3A

The atomic coordinates of the Cry3A protein are given in the Appendix included in Section 9.2

5.34 Example 34

Modification of Cry Genes for Expression in Plants

Wild-type cry genes are known to be expressed poorly in plants as a full length gene or as a truncated gene. Typically, the G+C content of a cry gene is low (37%) and often contains many A+T rich regions, potential polyadenylation sites and numerous ATTTA sequences. Table 25 shows a list of potential polyadenylation sequences which should be avoided when preparing the "plantized" gene construct.

TABLE 25

LIST OF SEQUENCES OF THE POTENTIAL
POLYADENYLATION SIGNALS

AATAAA*	AAGCAT
AATAAT*	AATAAT
AACCAA	ATACAT
ATATAA	AAAATA
AATCAA	ATTAAA**
ATACTA	AATTAA**
ATAAAA	AATACA**
ATGAAA	CATAAA**

*indicates a potential major plant polyadenylation site.

**indicates a potential minor animal polyadenylation site.

All others are potential minor plant polyadenylation sites.

The regions for mutagenesis may be selected in the following manner. All regions of the DNA sequence of the cry gene are identified which contained five or more consecutive base pairs which were A or T. These were ranked in terms of length and highest percentage of A+T in the surrounding sequence over a 20-30 base pair region. The DNA is analysed for regions which might contain polyadenylation sites or ATTTA sequences. Oligonucleotides are then designed which maximize the elimination of A+T consecutive regions which contained one or more polyadenylation sites or ATTTA sequences. Two potential plant polyadenylation sites have been shown to be more critical based on published reports. Codons are selected which increase G+C content, but do not generate restriction sites for enzymes useful for cloning and assembly of the modified gene (e.g. BamHI, BglII, SacI, NcoI, EcoRV, etc.). Likewise condons are avoided which contain the doublets TA or GC which have been reported to be infrequently-found codons in plants.

Although the CaMV35S promoter is generally a high level constitutive promoter in most plant tissues, the expression level of genes driven the CaMV35S promoter is low in floral tissue relative to the levels seen in leaf tissue. Because the economically important targets damaged by some insects are the floral parts or derived from floral parts (e.g., cotton squares and bolls, tobacco buds, tomato buds and fruit), it is often advantageous to increase the expression of crystal proteins in these tissues over that obtained with the CaMV35S promoter.

The 35S promoter of Figwort Mosaic Virus (FMV) is analogous to the CaMV35S promoter. This promoter has been isolated and engineered into a plant transformation vector. Relative to the CaMV promoter, the FMV 35S promoter is highly expressed in the floral tissue, while still providing similar high levels of gene expression in other tissues such as leaf. A plant transformation vector, may be constructed in which the full length synthetic cry gene is driven by the FMV 35S promoter. Tobacco plants may be transformed with the vector and compared for expression of the crystal protein by Western blot or ELISA immunoassay in leaf and floral tissue. The FMV promoter has been used to produce relatively high levels of crystal protein in floral tissue compared to the CaMV promoter.

5.35 Example 35

Expression of Synthetic Cry Genes with
ssRUBISCO Promoters and Chloroplast Transit
Peptides

The genes in plants encoding the small subunit of RUBISCO (SSU) are often highly expressed, light regulated

and sometimes show tissue specificity. These expression properties are largely due to the promoter sequences of these genes. It has been possible to use SSU promoters to express heterologous genes in transformed plants. Typically a plant will contain multiple SSU genes, and the expression levels and tissue specificity of different SSU genes will be different. The SSU proteins are encoded in the nucleus and synthesized in the cytoplasm as precursors that contain an N-terminal extension known as the chloroplast transit peptide (CTP). The CTP directs the precursor to the chloroplast and promotes the uptake of the SSU protein into the chloroplast. In this process, the CTP is cleaved from the SSU protein. These CTP sequences have been used to direct heterologous proteins into chloroplasts of transformed plants.

The SSU promoters might have several advantages for expression of heterologous genes in plants. Some SSU promoters are very highly expressed and could give rise to expression levels as high or higher than those observed with the CaMV35S promoter. The tissue distribution of expression from SSU promoters is different from that of the CaMV35S promoter, so for control of some insect pests, it may be advantageous to direct the expression of crystal proteins to those cells in which SSU is most highly expressed. For example, although relatively constitutive, in the leaf the CaMV35S promoter is more highly expressed in vascular tissue than in some other parts of the leaf, while most SSU promoters are most highly expressed in the mesophyll cells of the leaf. Some SSU promoters also are more highly tissue specific, so it could be possible to utilize a specific SSU promoter to express the protein of the present invention in only a subset of plant tissues, if for example expression of such a protein in certain cells was found to be deleterious to those cells. For example, for control of Colorado potato beetle in potato, it may be advantageous to use SSU promoters to direct crystal protein expression to the leaves but not to the edible tubers.

Utilizing SSU CTP sequences to localize crystal proteins to the chloroplast might also be advantageous. Localization of the *B. thuringiensis* crystal proteins to the chloroplast could protect these from proteases found in the cytoplasm. This could stabilize the proteins and lead to higher levels of accumulation of active toxin, cry genes containing the CTP could be used in combination with the SSU promoter or with other promoters such as CaMV35S.

5.36 Example 36

Targeting of Cry* Proteins to the Extracellular
Space or Cacuole through the Use of Signal
Peptides

The *B. thuringiensis* proteins produced from the synthetic genes described here are localized to the cytoplasm of the plant cell, and this cytoplasmic localization results in plants that are insecticidally effective. It may be advantageous for some purposes to direct the *B. thuringiensis* proteins to other compartments of the plant cell. Localizing *B. thuringiensis* proteins in compartments other than the cytoplasm may result in less exposure of the *B. thuringiensis* proteins to cytoplasmic proteases leading to greater accumulation of the protein yielding enhanced insecticidal activity. Extracellular localization could lead to more efficient exposure of certain insects to the *B. thuringiensis* proteins leading to greater efficacy. If a *B. thuringiensis* protein were found to be deleterious to plant cell function, then localization to a noncytoplasmic compartment could protect these cells from the protein.

In plants as well as other eukaryotes, proteins that are destined to be localized either extracellularly or in several specific compartments are typically synthesized with an N-terminal amino acid extension known as the signal peptide. The signal peptide directs the protein to enter the compartmentalization pathway, and it is typically cleaved from the mature protein as an early step in compartmentalization. For an extracellular protein, the secretory pathway typically involves cotranslational insertion into the endoplasmic reticulum with cleavage of the signal peptide occurring at this stage. The mature protein then passes through the Golgi body into vesicles that fuse with the plasma membrane thus releasing the protein into the extracellular space. Proteins destined for other compartments follow a similar pathway. For example, proteins that are destined for the endoplasmic reticulum or the Golgi body follow this scheme, but they are specifically retained in the appropriate compartment. In plants, some proteins are also targeted to the vacuole, another membrane bound compartment in the cytoplasm of many plant cells. Vacuole targeted proteins diverge from the above pathway at the Golgi body where they enter vesicles that fuse with the vacuole.

A common feature of this protein targeting is the signal peptide that initiates the compartmentalization process. Fusing a signal peptide to a protein will in many cases lead to the targeting of that protein to the endoplasmic reticulum. The efficiency of this step may depend on the sequence of the mature protein itself as well. The signals that direct a protein to a specific compartment rather than to the extracellular space are not as clearly defined. It appears that many of the signals that direct the protein to specific compartments are contained within the amino acid sequence of the mature protein. This has been shown for some vacuole targeted proteins, but it is not yet possible to define these sequences precisely. It appears that secretion into the extracellular space is the "default" pathway for a protein that contains a signal sequence but no other compartmentalization signals. Thus, a strategy to direct *B. thuringiensis* proteins out of the cytoplasm is to fuse the genes for synthetic *B. thuringiensis* genes to DNA sequences encoding known plant signal peptides. These fusion genes will give rise to *B. thuringiensis* proteins that enter the secretory pathway, and lead to extracellular secretion or targeting to the vacuole or other compartments. Signal sequences for several plant genes have been described. One such sequence is for the tobacco pathogenesis related protein PR1b has been previously described (Comelissen et al, 1986). The PR1b protein is normally localized to the extracellular space. Another type of signal peptide is contained on seed storage proteins of legumes. These proteins are localized to the protein body of seeds, which is a vacuole like compartment found in seeds. A signal peptide DNA sequence for the β -subunit of the 7S storage protein of common bean (*Phaseolus vulgaris*), PvuB has been described (Doyle et al, 1986). Based on the published these published sequences, genes may be synthesized chemically using oligonucleotides that encode the signal peptides for PR1b and PvuB. In some cases to achieve secretion or compartmentalization of heterologous proteins, it may be necessary to include some amino acid sequence beyond the normal cleavage site of the signal peptide. This may be necessary to insure proper cleavage of the signal peptide.

5.37 Example 37

Isolation of Transgenic Maize Resistant to Diabrotica spp. Using Cry3Bb Variants

5.37.1 Plant Gene Construction

The expression of a plant gene which exists in double-stranded DNA form involves transcription of messenger

RNA (mRNA) from one strand of the DNA by RNA polymerase enzyme, and the subsequent processing of the mRNA primary transcript inside the nucleus. This processing involves a 3' non-translated region which adds polyadenylate nucleotides to the 3' end of the RNA. Transcription of DNA into mRNA is regulated by a region of DNA usually referred to as the "promoter". The promoter region contains a sequence of bases that signals RNA polymerase to associate with the DNA and to initiate the transcription of mRNA using one of the DNA strands as a template to make a corresponding strand of RNA.

A number of promoters which are active in plant cells have been described in the literature. Such promoters may be obtained from plants or plant viruses and include, but are not limited to, the nopaline synthase (NOS) and octopine synthase (OCS) promoters (which are carried on tumor-inducing plasmids of *Agrobacterium tumefaciens*), the cauliflower mosaic virus (CaMV) 19S and 35S promoters, the light-inducible promoter from the small subunit of ribulose 1,5-bisphosphate carboxylase (ssRUBISCO, a very abundant plant polypeptide), and the Figwort Mosaic Virus (FMV) 35S promoter. All of these promoters have been used to create various types of DNA constructs which have been expressed in plants (see e.g., U.S. Pat. No. 5,463,175, specifically incorporated herein by reference).

The particular promoter selected should be capable of causing sufficient expression of the enzyme coding sequence to result in the production of an effective amount of protein. One set of preferred promoters are constitutive promoters such as the CaMV35S or FMV35S promoters that yield high levels of expression in most plant organs (U.S. Pat. No. 5,378,619, specifically incorporated herein by reference). Another set of preferred promoters are root enhanced or specific promoters such as the CaMV derived 4 as-1 promoter or the wheat POX1 promoter (U.S. Pat. No. 5,023,179, specifically incorporated herein by reference; Hertig et al., 1991). The root enhanced or specific promoters would be particularly preferred for the control of corn rootworm (*Diabrotica* spp.) in transgenic corn plants.

The promoters used in the DNA constructs (i.e. chimeric plant genes) of the present invention may be modified, if desired, to affect their control characteristics. For example, the CaMV35S promoter may be ligated to the portion of the ssRUBISCO gene that represses the expression of ssRUBISCO in the absence of light, to create a promoter which is active in leaves but not in roots. The resulting chimeric promoter may be used as described herein. For purposes of this description, the phrase "CaMV35S" promoter thus includes variations of CaMV35S promoter, e.g., promoters derived by means of ligation with operator regions, random or controlled mutagenesis, etc. Furthermore, the promoters may be altered to contain multiple "enhancer sequences" to assist in elevating gene expression.

The RNA produced by a DNA construct of the present invention also contains a 5' non-translated leader sequence. This sequence can be derived from the promoter selected to express the gene, and can be specifically modified so as to increase translation of the mRNA. The 5' non-translated regions can also be obtained from viral RNA's, from suitable eucaryotic genes, or from a synthetic gene sequence. The present invention is not limited to constructs wherein the non-translated region is derived from the 5' non-translated sequence that accompanies the promoter sequence.

For optimized expression in monocotyledonous plants such as maize, an intron should also be included in the DNA

expression construct. This intron would typically be placed near the 5' end of the mRNA in untranslated sequence. This intron could be obtained from, but not limited to, a set of introns consisting of the maize hsp70 intron (U.S. Pat. No. 5,424,412; specifically incorporated herein by reference) or the rice Act1 intron (McElroy et al., 1990). As shown below, the maize hsp70 intron is useful in the present invention.

As noted above, the 3' non-translated region of the chimeric plant genes of the present invention contains a polyadenylation signal which functions in plants to cause the addition of adenylate nucleotides to the 3' end of the RNA. Examples of preferred 3' regions are (1) the 3' transcribed, non-translated regions containing the polyadenylate signal of *Agrobacterium* tumor-inducing (Ti) plasmid genes, such as the nopaline synthase (NOS) gene and (2) plant genes such as the pea ssRUBISCO E9 gene (Fischhoff et al., 1987).

5.37.2 Plant Transformation and Expression

A chimeric plant gene containing a structural coding sequence of the present invention can be inserted into the genome of a plant by any suitable method. Suitable plant transformation vectors include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed, e.g., by Herrera-Estrella (1983), Bevan (1983), Klee (1985) and Eur. Pat. Appl. Publ. No. EP0120516. In addition to plant transformation vectors derived from the Ti or root-inducing (Ri) plasmids of *Agrobacterium*, alternative methods can be used to insert the DNA constructs of this invention into plant cells. Such methods may involve, for example, the use of liposomes, electroporation, chemicals that increase free DNA uptake, free DNA delivery via microprojectile bombardment, and transformation using viruses or pollen (Fromm et al, 1986; Armstrong et al., 1990; From et al., 1990).

5.37.3 Construction of Monocot Plant Expression Vectors for Cry3Bb Variants

5.37.3.1 Design of Cry3Bb Variant Genes for Plant Expression

For efficient expression of the cry3Bb variants in transgenic plants, the gene encoding the variants must have a suitable sequence composition (Diehn et al., 1996). One example of such a sequence is shown for the v11231 gene (SEQ ID NO:99) which encodes the Cry3Bb11231 variant protein (SEQ ID NO:100) with Diabrotica activity. This gene was derived via mutagenesis (Kunkel, 1985) of a cry3Bb synthetic gene (SEQ ID NO:101) encoding a protein essentially homologous to the protein encoded by the native cry3Bb gene (Gen Bank Accession Number m89794, SEQ ID NO:102). The following oligonucleotides were used in the mutagenesis of the original cry3Bb synthetic gene (SEQ ID NO:101) to create the v11231 gene (SEQ ID NO:99):

Oligo #1: (SEQ ID NO:103)

5'-TAGGCCTCCATCCATGGCAAACCCTAACAATC-3'

Oligo #2: (SEQ ID NO:104)

5'-TCCCATCTTCCTACTTACGACCCTGCA-GAAATACGGTCCAAC-3'

Oligo #3: (SEQ ID NO:105)

5'-GACCTCACCTACCAAACATTCGATCTTG-3'

Oligo #4:

(SEQ ID NO:106)

5'-CGAGTTCTACCGTAGGCAGCTCAAG-3'

5.37.3.2 Construction of Cry3Bb Monocot Plant Expression Vector

To place the cry3Bb variant gene v11231 in a vector suitable for expression in monocotyledonous plants (i.e. under control of the enhanced Cauliflower Mosaic Virus 35S promoter and link to the hsp70 intron followed by a nopaline synthase polyadenylation site as in U.S. Pat. No. 5,424,412, specifically incorporated herein by reference), the vector pMON19469 was digested with NcoI and EcoRI. The larger vector band of approximately 4.6 kb was electrophoresed, purified, and ligated with T4 DNA ligase to the NcoI-EcoRI fragment of approximately 2 kb containing the v11231 gene (SEQ ID NO:99). The ligation mix was transformed into *E. coli*, carbenicillin resistant colonies recovered and plasmid DNA recovered by DNA miniprep procedures. This DNA was subjected to restriction endonuclease analysis with enzymes such as NcoI and EcoRI (together), NotI, and PstI to identify clones containing pMON33708 (the v11231 coding sequence fused to the hsp70 intron under control of the enhanced CaMV35S promoter).

To place the v11231 gene in a vector suitable for recovery of stably transformed and insect resistant plants, the 3.75-kb NotI restriction fragment from pMON33708 containing the v11231 coding sequence fused to the hsp70 intron under control of the enhanced CaMV35S promoter was isolated by gel electrophoresis and purification. This fragment was ligated with pMON30460 treated with NotI and calf intestinal alkaline phosphatase (pMON30460 contains the neomycin phosphotransferase coding sequence under control of the CaMV35S promoter). Kanamycin resistant colonies were obtained by transformation of this ligation mix into *E. coli* and colonies containing pMON33710 identified by restriction endonuclease digestion of plasmid miniprep DNAs. Restriction enzymes such as NotI, EcoRV, HindIII, NcoI, EcoRI, and BglII can be used to identify the appropriate clones containing the NotI fragment of pMON33708 in the NotI site of pMON30460 (i.e. pMON33710) in the orientation such that both genes are in tandem (i.e. the 3' end of the v11231 expression cassette is linked to the 5' end of the nptII expression cassette). Expression of the v11231 protein by pMON33710 in corn protoplasts was confirmed by electroporation of pMON33710 DNA into protoplasts followed by protein blot and ELISA analysis. This vector can be introduced into the genomic DNA of corn embryos by particle gun bombardment followed by paromomycin selection to obtain corn plants expressing the v11231 gene essentially as described in U.S. Pat. No. 5,424,412, specifically incorporated herein by reference.

In this example, the vector was introduced via cobombardment with a hygromycin resistance conferring plasmid into immature embryo scutella (IES) of maize, followed by hygromycin selection, and regeneration. Transgenic corn lines expressing the v11231 protein were identified by ELISA analysis. Progeny seed from these events were subsequently tested for protection from *Diabrotica* feeding.

5.37.3.3 In Planta Performance of Cry3Bb.11231

Transformed corn plants expressing Cry3Bb.11231 protein were challenged with western corn rootworm (WCR) larvae in both a seedling and 10 inch pot assay. The transformed genotype was A634, where the progeny of the RO cross by A634 was evaluated. Observations included effect on larval development (weight), root damage rating

(RDR), and protein expression. The transformation vector containing the cry3Bb gene was pMON33710. Treatments included the positive and negative iso-populations for each event and an A634 check.

The seedling assay consisted of the following steps: (i) single seeds were placed in 1 oz cups containing potting soil; (ii) at spiking, each seedling was infested with 4 neonate larvae; and (iii) after infestation, seedlings were incubated for 7 days at 25° C., 50% RH, and 14:10 (L:D) photo period. Adequate moisture was added to the potting soil during the incubation period to maintain seedling vigor.

The 10 inch pot assay consisted of the following steps: (i) single seeds were placed in 10 inch pots containing potting soil, (ii) at 14 days post planting, each pot was infested with 800 eggs which have been pre-incubated such that hatch would occur 5–7 days post infestation; and (iii) after infestation, plants were incubated for 4 weeks under the same environmental conditions as the seedling assay. Pots were both sub and top irrigated daily.

For the seedling assay, on day 7 plants were given a root damage rating, and surviving larvae were weighed. Also at this time, Cry3Bb protein concentrations in the roots were determined by ELISA. The scale used for the seedling assay to assess root damage is as follows: RDR (root damage rating) 0=no visible feeding; RDR 1=very light feeding; RDR 2=light feeding; RDR 3=moderate feeding; RDR 4=heavy feeding; and RDR 5=very heavy feeding.

Results of the seedling assay are shown in Table 26. Plants expressing Cry3Bb protein were completely protected by WCR feeding, where surviving larvae within this treatment had not grown. Mean larval weights ranged from 2.03–2.73 mg for the nonexpressing treatments, where the surviving larval average weight was 0.11 mg on the expressing cry3Bb treatment. Root damage ratings were 3.86 and 0.33 for the nonexpressing and expressing isopopulations, respectively. Larval survival ranged from 75–85% for the negative and check treatments, where only 25% of the larvae survived on the Cry3Bb treatment.

TABLE 26

EFFECT OF CRY3BB EXPRESSING PLANTS ON WCR LARVAE IN A SEEDLING ASSAY							
					Larvae		
		Plants			Mean ± SD		
Event	Treatment	N	Root (ppm)	RDR ± SD	N	% Surv	Wt. (mg)
16	Negative	7	0.0	3.86 ± 0.65	21	75	2.73 ± 1.67
16	Positive	3	29.01	0.33 ± 0.45	3	25	0.11 ± 0.07
A634	Check	4	0.0	—	13	81	2.03 ± 0.83

For the 10 inch pot assay, at 4 weeks post infestation plant height was recorded and a root damage rating (Iowa 1–6 scale; Hills and Peters, 1971) was given.

Results of the 10 inch pot assay are shown in Table 27. Plants expressing Cry3Bb protein had significantly less feeding damage and were taller than the non-expressing plants. Event 16, the higher of the two expressing events provided nearly complete control. The negative treatments had very high root damage ratings indicating very high insect pressure. The positive mean root damage ratings were 3.4 and 2.2 for event 6 and 16, respectively. Mean RDR for the negative treatment was 5.0 and 5.6.

TABLE 27

EFFECT OF CRY3BB EXPRESSING CORN IN CONTROLLING WCR LARVAL FEEDING IN A 10 INCH POT ASSAY					
Event	Treatment	N	Root (ppm)	RDR ± SD	Plant Height (cm)
6	Negative	7	0.0	5.0 ± 1.41	49.7 ± 18.72
6	Positive	5	7.0	3.4 ± 1.14	73.9 ± 8.67
16	Negative	5	0.0	5.6 ± 0.89	61.2 ± 7.75
16	Positive	5	55.0	2.2 ± 0.84	83.8 ± 7.15

In summary, corn plants expressing Cry3Bb protein have a significant biological effect on WCR larval development as seen in the seedling assay. When challenged with very high infestation levels, plants expressing the Cry3Bb protein were protected from WCR larval feeding damage as illustrated in the 10 inch pot assay.

6.0 BRIEF DESCRIPTION OF THE SEQUENCE IDENTIFIERS

SEQ ID NO:1 DNA sequence of cry3Bb.11221 gene.
 SEQ ID NO:2 Amino acid sequence of Cry3Bb.11221 polypeptide.
 SEQ ID NO:3 DNA sequence of cry3Bb.11222 gene.
 SEQ ID NO:4 Amino acid sequence of Cry3Bb.11222 polypeptide.
 SEQ ID NO:5 DNA sequence of cry3Bb.11223 gene.
 SEQ ID NO:6 Amino acid sequence of Cry3Bb.11223 polypeptide.
 SEQ ID NO:7 DNA sequence of cry3Bb.11224 gene.
 SEQ ID NO:8 Amino acid sequence of Cry3Bb.11224 polypeptide.
 SEQ ID NO:9 DNA sequence of cry3Bb.11225 gene.
 SEQ ID NO:10 Amino acid sequence of Cry3Bb.11225 polypeptide.
 SEQ ID NO:11 DNA sequence of cry3Bb.11226 gene.
 SEQ ID NO:12 Amino acid sequence of Cry3Bb.11226 polypeptide.
 SEQ ID NO:13 DNA sequence of cry3Bb.11227 gene.
 SEQ ID NO:14 Amino acid sequence of Cry3Bb.11227 polypeptide.
 SEQ ID NO:15 DNA sequence of cry3Bb.11228 gene.
 SEQ ID NO:16 Amino acid sequence of Cry3Bb.11228 polypeptide.
 SEQ ID NO:17 DNA sequence of cry3Bb.11229 gene.
 SEQ ID NO:18 Amino acid sequence of Cry3Bb.11229 polypeptide.
 SEQ ID NO:19 DNA sequence of cry3Bb.11230 gene.
 SEQ ID NO:20 Amino acid sequence of Cry3Bb.11230 polypeptide.
 SEQ ID NO:21 DNA sequence of cry3Bb.11231 gene.
 SEQ ID NO:22 Amino acid sequence of Cry3Bb.11231 polypeptide.
 SEQ ID NO:23 DNA sequence of cry3Bb.11232 gene.
 SEQ ID NO:24 Amino acid sequence of Cry3Bb.11232 polypeptide.
 SEQ ID NO:25 DNA sequence of cry3Bb.11233 gene.
 SEQ ID NO:26 Amino acid sequence of Cry3Bb.11233 polypeptide.
 SEQ ID NO:27 DNA sequence of cry3Bb.11234 gene.
 SEQ ID NO:28 Amino acid sequence of Cry3Bb.11234 polypeptide.
 SEQ ID NO:29 DNA sequence of cry3Bb.11235 gene.
 SEQ ID NO:30 Amino acid sequence of Cry3Bb.11235 polypeptide.
 SEQ ID NO:31 DNA sequence of cry3Bb.11236 gene.

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SEQ ID NO:32 Amino acid sequence of Cry3Bb.11236 polypeptide.
 SEQ ID NO:33 DNA sequence of cry3Bb.11237 gene.
 SEQ ID NO:34 Amino acid sequence of Cry3Bb.11237 polypeptide.
 SEQ ID NO:35 DNA sequence of cry3Bb.11238 gene.
 SEQ ID NO:36 Amino acid sequence of Cry3Bb.11238 polypeptide.
 SEQ ID NO:37 DNA sequence of cry3Bb.11239 gene.
 SEQ ID NO:38 Amino acid sequence of Cry3Bb.11239 polypeptide.
 SEQ ID NO:39 DNA sequence of cry3Bb.11241 gene.
 SEQ ID NO:40 Amino acid sequence of Cry3Bb.11241 polypeptide.
 SEQ ID NO:41 DNA sequence of cry3Bb.11242 gene.
 SEQ ID NO:42 Amino acid sequence of Cry3Bb.11242 polypeptide.
 SEQ ID NO:43 DNA sequence of cry3Bb.11032 gene.
 SEQ ID NO:44 Amino acid sequence of Cry3Bb.11032 polypeptide.
 SEQ ID NO:45 DNA sequence of cry3Bb.11035 gene.
 SEQ ID NO:46 Amino acid sequence of Cry3Bb.11035 polypeptide.
 SEQ ID NO:47 DNA sequence of cry3Bb.11036 gene.
 SEQ ID NO:48 Amino acid sequence of Cry3Bb.11036 polypeptide.
 SEQ ID NO:49 DNA sequence of cry3Bb.11046 gene.
 SEQ ID NO:50 Amino acid sequence of Cry3Bb.11046 polypeptide.
 SEQ ID NO:51 DNA sequence of cry3Bb.11048 gene.
 SEQ ID NO:52 Amino acid sequence of Cry3Bb.11048 polypeptide.
 SEQ ID NO:53 DNA sequence of cry3Bb.11051 gene.
 SEQ ID NO:54 Amino acid sequence of Cry3Bb.11051 polypeptide.
 SEQ ID NO:55 DNA sequence of cry3Bb.11057 gene.
 SEQ ID NO:56 Amino acid sequence of Cry3Bb.11057 polypeptide.
 SEQ ID NO:57 DNA sequence of cry3Bb.11058 gene.
 SEQ ID NO:58 Amino acid sequence of Cry3Bb.11058 polypeptide.
 SEQ ID NO:59 DNA sequence of cry3Bb.11081 gene.
 SEQ ID NO:60 Amino acid sequence of Cry3Bb.11081 polypeptide.
 SEQ ID NO:61 DNA sequence of cry3Bb.11082 gene.
 SEQ ID NO:62 Amino acid sequence of Cry3Bb.11082 polypeptide.
 SEQ ID NO:63 DNA sequence of cry3Bb.11083 gene.
 SEQ ID NO:64 Amino acid sequence of Cry3Bb.11083 polypeptide.
 SEQ ID NO:65 DNA sequence of cry3Bb.11084 gene.
 SEQ ID NO:66 Amino acid sequence of Cry3Bb.11084 polypeptide.
 SEQ ID NO:67 DNA sequence of cry3Bb.11095 gene.
 SEQ ID NO:68 Amino acid sequence of Cry3Bb.11095 polypeptide.
 SEQ ID NO:69 DNA sequence of cry3Bb.60 gene.
 SEQ ID NO:70 Amino acid sequence of Cry3Bb.60 polypeptide.
 SEQ ID NO:71 Primer FW001.
 SEQ ID NO:72 Primer FW006.
 SEQ ID NO:73 Primer MVT095.
 SEQ ID NO:74 Primer MVT097.
 SEQ ID NO:75 Primer MVT091.
 SEQ ID NO:76 Primer MVT075.
 SEQ ID NO:77 Primer MVT076.
 SEQ ID NO:78 Primer MVT111.

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SEQ ID NO:79 Primer MVT094.
 SEQ ID NO:80 Primer MVT103.
 SEQ ID NO:81 Primer MVT081.
 SEQ ID NO:82 Primer MVT085.
 5 SEQ ID NO:83 Primer A.
 SEQ ID NO:84 Primer B.
 SEQ ID NO:85 Primer C.
 SEQ ID NO:86 Primer D.
 SEQ ID NO:87 Primer E.
 10 SEQ ID NO:88 Primer F.
 SEQ ID NO:89 Primer G.
 SEQ ID NO:90 Primer WD112.
 SEQ ID NO:91 Primer WD115.
 SEQ ID NO:92 Primer MVT105.
 15 SEQ ID NO:93 Primer MVT092.
 SEQ ID NO:94 Primer MVT070.
 SEQ ID NO:95 Primer MVT083.
 SEQ ID NO:96 N-terminal amino acid of Cry3Bb polypeptide.
 20 SEQ ID NO:97 DNA sequence of wild-type cry3Bb gene.
 SEQ ID NO:98 Amino acid sequence of wild-type Cry3Bb polypeptide.
 SEQ ID NO:99 Plantized DNA sequence for cry3Bb.11231 gene.
 25 SEQ ID NO:100 Amino acid sequence of plantized Cry3Bb.11231 polypeptide.
 SEQ ID NO:101 DNA sequence of cry3Bb gene used to prepare SEQ ID NO:99.
 SEQ ID NO:102 DNA sequence of wild-type cry3Bb gene,
 30 Genbank #M89794.
 SEQ ID NO:103 DNA sequence of Oligo #1.
 SEQ ID NO:104 DNA sequence of Oligo #2.
 SEQ ID NO:105 DNA sequence of Oligo #3.
 SEQ ID NO:106 DNA sequence of Oligo #4.
 35 SEQ ID NO:107 DNA sequence of cry3Bb.11098 gene.
 SEQ ID NO:108 Amino acid sequence of Cry3Bb.11098 polypeptide.

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 All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta				624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	195	200	205	
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa				672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	210	215	220	
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac				720
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr	225	230	235	240
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga				768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	245	250	255	
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa				816
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	260	265	270	
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat				864
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	275	280	285	
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att				912
Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	290	295	300	
ttt acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca				960
Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro	305	310	315	320
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat				1008
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	325	330	335	
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt				1056
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe	340	345	350	
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga				1104
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg	355	360	365	
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat				1152
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp	370	375	380	
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt				1200

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Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val		
385					390					395					400		
tat	cga	act	ata	gct	aat	aca	gac	gta	gcg	gct	tggt	ccg	aat	ggt	aag	1248	
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys		
			405					410						415			
gta	tat	tta	ggt	gtt	acg	aaa	gtt	gat	ttt	agt	caa	tat	gat	gat	caa	1296	
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln		
			420				425						430				
aaa	aat	gaa	act	agt	aca	caa	aca	tat	gat	tca	aaa	aga	aac	aat	ggc	1344	
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly		
		435					440					445					
cat	gta	agt	gca	cag	gat	tct	att	gac	caa	tta	ccg	cca	gaa	aca	aca	1392	
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr		
	450				455					460							
gat	gaa	cca	ctt	gaa	aaa	gca	tat	agt	cat	cag	ctt	aat	tac	gcg	gaa	1440	
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu		
	465				470					475					480		
tgt	ttc	tta	atg	cag	gac	cgt	cgt	gga	aca	att	cca	ttt	ttt	act	tggt	1488	
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp		
			485					490						495			
aca	cat	aga	agt	gta	gac	ttt	ttt	aat	aca	att	gat	gct	gaa	aag	att	1536	
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile		
			500					505					510				
act	caa	ctt	cca	gta	gtg	aaa	gca	tat	gcc	ttg	tct	tca	ggt	gct	tcc	1584	
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser		
		515					520					525					
att	att	gaa	ggt	cca	gga	ttc	aca	gga	gga	aat	tta	cta	ttc	cta	aaa	1632	
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys		
		530					535				540						
gaa	tct	agt	aat	tca	att	gct	aaa	ttt	aaa	gtt	aca	tta	aat	tca	gca	1680	
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala		
	545				550					555					560		
gcc	ttg	tta	caa	cga	tat	cgt	gta	aga	ata	cgc	tat	gct	tct	acc	act	1728	
Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr		
			565						570					575			
aac	tta	cga	ctt	ttt	gtg	caa	aat	tca	aac	aat	gat	ttt	ctt	gtc	atc	1776	
Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile		
		580						585					590				
tac	att	aat	aaa	act	atg	aat	aaa	gat	gat	gat	tta	aca	tat	caa	aca	1824	
Tyr	Ile	Asn	Lys	Thr	Met	Asn	Lys	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr		
		595					600					605					
ttt	gat	ctc	gca	act	act	aat	tct	aat	atg	ggg	ttc	tcg	ggt	gat	aag	1872	
Phe	Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys		
		610					615						620				
aat	gaa	ctt	ata	ata	gga	gca	gaa	tct	ttc	gtt	tct	aat	gaa	aaa	atc	1920	
Asn	Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile		
	625					630				635					640		
tat	ata	gat	aag	ata	gaa	ttt	atc	cca	gta	caa	ttg	taa				1959	
Tyr	Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu						
			645						650								

<210> SEQ ID NO 2

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 2

Met	Asn	Pro	Asn	Asn	Arg	Ser	Glu	His	Asp	Thr	Ile	Lys	Val	Thr	Pro
1			5						10					15	

Asn	Ser	Glu	Leu	Gln	Thr	Asn	His	Asn	Gln	Tyr	Pro	Leu	Ala	Asp	Asn	
			20				25						30			
Pro	Asn	Ser	Thr	Leu	Glu	Glu	Leu	Asn	Tyr	Lys	Glu	Phe	Leu	Arg	Met	
			35				40						45			
Thr	Glu	Asp	Ser	Ser	Thr	Glu	Val	Leu	Asp	Asn	Ser	Thr	Val	Lys	Asp	
			50				55						60			
Ala	Val	Gly	Thr	Gly	Ile	Ser	Val	Val	Gly	Gln	Ile	Leu	Gly	Val	Val	
			65				70						75			
Gly	Val	Pro	Phe	Ala	Gly	Ala	Leu	Thr	Ser	Phe	Tyr	Gln	Ser	Phe	Leu	
			85				90						95			
Asn	Thr	Ile	Trp	Pro	Ser	Asp	Ala	Asp	Pro	Trp	Lys	Ala	Phe	Met	Ala	
			100				105						110			
Gln	Val	Glu	Val	Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser	
			115				120						125			
Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr	
			130				135						140			
Val	Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Phe	His	His	Ser	Arg	Arg	Ser	
			145				150						155			
Lys	Arg	Ser	Gln	Asp	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser	
			165				170						175			
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val	
			180				185						190			
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	
			195				200						205			
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu	
			210				215						220			
Asp	Val	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr	
			225				230						235			
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg	
			245				250						255			
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu	
			260				265						270			
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp	
			275				280						285			
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile	
			290				295						300			
Phe	Thr	Asp	Pro	Ile	Phe	Ser	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro	
			305				310						315			
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	
			325				330						335			
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe	
			340				345						350			
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg	
			355				360						365			
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp	
			370				375						380			
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val	
			385				390						395			
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys	
			405				410						415			
Val	Tyr	Leu														

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Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly
 435 440 445
 His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr
 450 455 460
 Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu
 465 470 475 480
 Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp
 485 490 495
 Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile
 500 505 510
 Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser
 515 520 525
 Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys
 530 535 540
 Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
 545 550 555 560
 Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
 565 570 575
 Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
 580 585 590
 Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
 595 600 605
 Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
 610 615 620
 Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
 625 630 635 640
 Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> SEQ ID NO 3
 <211> LENGTH: 1959
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant delta endotoxin
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1956)

<400> SEQUENCE: 3

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct	48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	

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aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala 100 105 110	336
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt Gln Val Gln Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser 115 120 125	384
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr 130 135 140	432
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser 145 150 155 160	480
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser 165 170 175	528
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val 180 185 190	576
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu 195 200 205	624
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu 210 215 220	672
gat gtt gct gaa ttc ctt agt aga caa tta aaa ctt aca caa caa tac Asp Val Ala Glu Phe Leu Ser Arg Gln Leu Lys Leu Thr Gln Gln Tyr 225 230 235 240	720
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg 245 250 255	768
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu 260 265 270	816
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp 275 280 285	864
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile 290 295 300	912
ttt acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro 305 310 315 320	960
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp 325 330 335	1008
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe 340 345 350	1056
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg 355 360 365	1104
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp 370 375 380	1152
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 385 390 395 400	1200
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys 405 410 415	1248

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gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa	1296
Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln	
420 425 430	
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc	1344
Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly	
435 440 445	
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca	1392
His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr	
450 455 460	
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa	1440
Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu	
465 470 475 480	
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg	1488
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp	
485 490 495	
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att	1536
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile	
500 505 510	
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc	1584
Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser	
515 520 525	
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa	1632
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys	
530 535 540	
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca	1680
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala	
545 550 555 560	
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act	1728
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr	
565 570 575	
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc	1776
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile	
580 585 590	
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca	1824
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr	
595 600 605	
ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag	1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys	
610 615 620	
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc	1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile	
625 630 635 640	
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa	1959
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu	
645 650	

<210> SEQ ID NO 4

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 4

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
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Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	

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Thr	Glu	Asp	Ser	Ser	Thr	Glu	Val	Leu	Asp	Asn	Ser	Thr	Val	Lys	Asp
50						55					60				
Ala	Val	Gly	Thr	Gly	Ile	Ser	Val	Val	Gly	Gln	Ile	Leu	Gly	Val	Val
65					70					75					80
Gly	Val	Pro	Phe	Ala	Gly	Ala	Leu	Thr	Ser	Phe	Tyr	Gln	Ser	Phe	Leu
				85					90					95	
Asn	Thr	Ile	Trp	Pro	Ser	Asp	Ala	Asp	Pro	Trp	Lys	Ala	Phe	Met	Ala
			100					105					110		
Gln	Val	Glu	Val	Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser
		115					120					125			
Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr
	130					135					140				
Val	Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Thr	Pro	Leu	Ser	Leu	Arg	Ser
145					150					155					160
Lys	Arg	Ser	Gln	Asp	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser
				165					170					175	
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val
			180					185					190		
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu
		195					200					205			
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu
	210					215					220				
Asp	Val	Ala	Glu	Phe	Leu	Ser	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr
225					230					235					240
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg
				245					250					255	
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu
			260					265					270		
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp
		275					280					285			
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile
	290					295					300				
Phe	Thr	Asp	Pro	Ile	Phe	Ser	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro
305					310					315					320
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp
				325					330					335	
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe
		340						345					350		
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg
		355					360					365			
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp
		370				375					380				
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val
385					390					395					400
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys
				405					410					415	
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln
		420						425					430		
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly
		435					440					445			
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr
	450					455					460				

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Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu
 465 470 475 480
 Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp
 485 490 495
 Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile
 500 505 510
 Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser
 515 520 525
 Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys
 530 535 540
 Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
 545 550 555 560
 Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
 565 570 575
 Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
 580 585 590
 Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
 595 600 605
 Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
 610 615 620
 Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
 625 630 635 640
 Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> SEQ ID NO 5
 <211> LENGTH: 1959
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant delta endotoxin
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1956)

<400> SEQUENCE: 5

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Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca	336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt	384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	

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115	120	125	
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr 130 135 140			432
ggt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser 145 150 155 160			480
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser 165 170 175			528
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val 180 185 190			576
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu 195 200 205			624
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct cca gaa Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Pro Glu 210 215 220			672
gat gtt gct gaa ttc agt cat aga caa tta aaa ctt aca caa caa tac Asp Val Ala Glu Phe Ser His Arg Gln Leu Lys Leu Thr Gln Gln Tyr 225 230 235 240			720
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg 245 250 255			768
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu 260 265 270			816
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp 275 280 285			864
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile 290 295 300			912
ttt acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro 305 310 315 320			960
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp 325 330 335			1008
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe 340 345 350			1056
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg 355 360 365			1104
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp 370 375 380			1152
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 385 390 395 400			1200
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys 405 410 415			1248
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln 420 425 430			1296
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc			1344

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Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly		
	435						440					445					
cat	gta	agt	gca	cag	gat	tct	att	gac	caa	tta	ccg	cca	gaa	aca	aca	1392	
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr		
	450					455				460							
gat	gaa	cca	ctt	gaa	aaa	gca	tat	agt	cat	cag	ctt	aat	tac	gcg	gaa	1440	
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu		
	465				470					475					480		
tgt	ttc	tta	atg	cag	gac	cgt	cgt	gga	aca	att	cca	ttt	ttt	act	tg	1488	
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp		
				485				490						495			
aca	cat	aga	agt	gta	gac	ttt	ttt	aat	aca	att	gat	gct	gaa	aag	att	1536	
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile		
			500					505					510				
act	caa	ctt	cca	gta	gtg	aaa	gca	tat	gcc	ttg	tct	tca	ggt	gct	tcc	1584	
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser		
		515				520						525					
att	att	gaa	ggt	cca	gga	ttc	aca	gga	gga	aat	tta	cta	ttc	cta	aaa	1632	
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys		
	530					535					540						
gaa	tct	agt	aat	tca	att	gct	aaa	ttt	aaa	gtt	aca	tta	aat	tca	gca	1680	
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala		
	545				550				555						560		
gcc	ttg	tta	caa	cga	tat	cgt	gta	aga	ata	cgc	tat	gct	tct	acc	act	1728	
Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr		
			565					570						575			
aac	tta	cga	ctt	ttt	gtg	caa	aat	tca	aac	aat	gat	ttt	ctt	gtc	atc	1776	
Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile		
		580						585					590				
tac	att	aat	aaa	act	atg	aat	aaa	gat	gat	gat	tta	aca	tat	caa	aca	1824	
Tyr	Ile	Asn	Lys	Thr	Met	Asn	Lys	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr		
	595					600						605					
ttt	gat	ctc	gca	act	act	aat	tct	aat	atg	ggg	ttc	tcg	ggt	gat	aag	1872	
Phe	Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys		
	610					615					620						
aat	gaa	ctt	ata	ata	gga	gca	gaa	tct	ttc	gtt	tct	aat	gaa	aaa	atc	1920	
Asn	Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile		
	625				630					635					640		
tat	ata	gat	aag	ata	gaa	ttt	atc	cca	gta	caa	ttg	taa				1959	
Tyr	Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu						
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<210> SEQ ID NO 6

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 6

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Asn	Ser	Glu	Leu	Gln	Thr	Asn	His	Asn	Gln	Tyr	Pro	Leu	Ala	Asp	Asn
		20						25					30		
Pro	Asn	Ser	Thr	Leu	Glu	Glu	Leu	Asn	Tyr	Lys	Glu	Phe	Leu	Arg	Met
		35					40					45			
Thr	Glu	Asp	Ser	Ser	Thr	Glu	Val	Leu	Asp	Asn	Ser	Thr	Val	Lys	Asp
	50					55					60				
Ala	Val	Gly	Thr	Gly	Ile	Ser	Val	Val	Gly	Gln	Ile	Leu	Gly	Val	Val
65					70					75					80

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Gly Val Pro Phe	Ala Gly	Ala Leu Thr	Ser Phe	Tyr Gln	Ser Phe	Leu	
	85		90		95		
Asn Thr Ile Trp	Pro Ser	Asp Ala	Asp Pro	Trp Lys	Ala Phe	Met Ala	
	100		105		110		
Gln Val Glu Val	Leu Ile	Asp Lys	Lys Ile	Glu Glu	Tyr Ala	Lys Ser	
	115		120		125		
Lys Ala Leu Ala	Glu Leu	Gln Gly	Leu Gln	Asn Asn	Phe Glu	Asp Tyr	
	130		135		140		
Val Asn Ala Leu	Asn Ser	Trp Lys	Lys Thr	Pro Leu	Ser Leu	Arg Ser	
	145		150		155		160
Lys Arg Ser Gln	Asp Arg	Ile Arg	Glu Leu	Phe Ser	Gln Ala	Glu Ser	
	165		170			175	
His Phe Arg Asn	Ser Met	Pro Ser	Phe Ala	Val Ser	Lys Phe	Glu Val	
	180		185		190		
Leu Phe Leu Pro	Thr Tyr	Ala Gln	Ala Ala	Asn Thr	His Leu	Leu Leu	
	195		200		205		
Leu Lys Asp Ala	Gln Val	Phe Gly	Glu Glu	Trp Gly	Tyr Ser	Pro Glu	
	210		215		220		
Asp Val Ala Glu	Phe Ser	His Arg	Gln Leu	Lys Leu	Thr Gln	Gln Tyr	
	225		230		235		240
Thr Asp His Cys	Val Asn	Trp Tyr	Asn Val	Gly Leu	Asn Gly	Leu Arg	
	245		250			255	
Gly Ser Thr Tyr	Asp Ala	Trp Val	Lys Phe	Asn Arg	Phe Arg	Arg Glu	
	260		265		270		
Met Thr Leu Thr	Val Leu	Asp Leu	Ile Val	Leu Phe	Pro Phe	Tyr Asp	
	275		280		285		
Ile Arg Leu Tyr	Ser Lys	Gly Val	Lys Thr	Glu Leu	Thr Arg	Asp Ile	
	290		295		300		
Phe Thr Asp Pro	Ile Phe	Ser Leu	Asn Thr	Leu Gln	Glu Tyr	Gly Pro	
	305		310		315		320
Thr Phe Leu Ser	Ile Glu	Asn Ser	Ile Arg	Lys Pro	His Leu	Phe Asp	
	325		330			335	
Tyr Leu Gln Gly	Ile Glu	Phe His	Thr Arg	Leu Gln	Pro Gly	Tyr Phe	
	340		345		350		
Gly Lys Asp Ser	Phe Asn	Tyr Trp	Ser Gly	Asn Tyr	Val Glu	Thr Arg	
	355		360		365		
Pro Ser Ile Gly	Ser Ser	Lys Thr	Ile Thr	Ser Pro	Phe Tyr	Gly Asp	
	370		375		380		
Lys Ser Thr Glu	Pro Val	Gln Lys	Leu Ser	Phe Asp	Gly Gln	Lys Val	
	385		390		395		400
Tyr Arg Thr Ile	Ala Asn	Thr Asp	Val Ala	Ala Trp	Pro Asn	Gly Lys	
	405		410			415	
Val Tyr Leu Gly	Val Thr	Lys Val	Asp Phe	Ser Gln	Tyr Asp	Asp Gln	
	420		425		430		
Lys Asn Glu Thr	Ser Thr	Gln Thr	Tyr Asp	Ser Lys	Arg Asn	Asn Gly	
	435		440		445		
His Val Ser Ala	Gln Asp	Ser Ile	Asp Gln	Leu Pro	Pro Glu	Thr Thr	
	450		455		460		
Asp Glu Pro Leu	Glu Lys	Ala Tyr	Ser His	Gln Leu	Asn Tyr	Ala Glu	
	465		470		475		480
Cys Phe Leu Met	Gln Asp	Arg Arg	Gly Thr	Ile Pro	Phe Phe	Thr Trp	
	485		490			495	

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Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile
500 505 510

Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser
515 520 525

Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys
530 535 540

Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
545 550 555 560

Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
565 570 575

Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
580 585 590

Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
595 600 605

Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
610 615 620

Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
625 630 635 640

Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
645 650

<210> SEQ ID NO 7
<211> LENGTH: 1959
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant delta endotoxin
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1956)

<400> SEQUENCE: 7

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aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca	336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt	384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat	432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	

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ggt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt	480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser	
145 150 155 160	
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt	528
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	
165 170 175	
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg	576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	
180 185 190	
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta	624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	
195 200 205	
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa	672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	
210 215 220	
gat gtt gct gaa ttc tat cgt aga caa tta aaa ctt aca caa caa tac	720
Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln Gln Tyr	
225 230 235 240	
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga	768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	
245 250 255	
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa	816
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	
260 265 270	
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat	864
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	
275 280 285	
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att	912
Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	
290 295 300	
ttt acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca	960
Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro	
305 310 315 320	
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat	1008
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	
325 330 335	
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt	1056
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe	
340 345 350	
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga	1104
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg	
355 360 365	
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat	1152
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp	
370 375 380	
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt	1200
Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val	
385 390 395 400	
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag	1248
Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys	
405 410 415	
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa	1296
Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln	
420 425 430	
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc	1344
Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly	
435 440 445	
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca	1392
His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr	
450 455 460	

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Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
 115 120 125
 Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
 130 135 140
 Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
 145 150 155 160
 Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser
 165 170 175
 His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val
 180 185 190
 Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
 195 200 205
 Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
 210 215 220
 Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln Gln Tyr
 225 230 235 240
 Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg
 245 250 255
 Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu
 260 265 270
 Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp
 275 280 285
 Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile
 290 295 300
 Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro
 305 310 315 320
 Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp
 325 330 335
 Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe
 340 345 350
 Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg
 355 360 365
 Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp
 370 375 380
 Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val
 385 390 395 400
 Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys
 405 410 415
 Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln
 420 425 430
 Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly
 435 440 445
 His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr
 450 455 460
 Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu
 465 470 475 480
 Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp
 485 490 495
 Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile
 500 505 510
 Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser
 515 520 525

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Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys
  530                               535                               540

Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
545                               550                               555                               560

Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
                               565                               570                               575

Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
                               580                               585                               590

Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
                               595                               600                               605

Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
  610                               615                               620

Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
625                               630                               635                               640

Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
                               645                               650

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<210> SEQ ID NO 9
<211> LENGTH: 1959
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant delta endotoxin
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1956)

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<400> SEQUENCE: 9

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atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct      48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
  1                               5                               10                               15

aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat      96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
                               20                               25                               30

cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg     144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
  35                               40                               45

act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat     192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
  50                               55                               60

gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta     240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
  65                               70                               75                               80

gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt     288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
  85                               90                               95

aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca     336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
  100                              105                              110

caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt     384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
  115                              120                              125

aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat     432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
  130                              135                              140

gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt     480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
  145                              150                              155                              160

aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt     528
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser

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[illegible]

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Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp	
			485						490					495		
aca	cat	aga	agt	gta	gac	ttt	ttt	aat	aca	att	gat	gct	gaa	aag	att	1536
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile	
			500					505					510			
act	caa	ctt	cca	gta	gtg	aaa	gca	tat	gcc	ttg	tct	tca	ggt	gct	tcc	1584
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser	
		515					520					525				
att	att	gaa	ggt	cca	gga	ttc	aca	gga	gga	aat	tta	cta	ttc	cta	aaa	1632
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys	
		530				535				540						
gaa	tct	agt	aat	tca	att	gct	aaa	ttt	aaa	gtt	aca	tta	aat	tca	gca	1680
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala	
545				550					555						560	
gcc	ttg	tta	caa	cga	tat	cgt	gta	aga	ata	cgc	tat	gct	tct	acc	act	1728
Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr	
			565					570					575			
aac	tta	cga	ctt	ttt	gtg	caa	aat	tca	aac	aat	gat	ttt	ctt	gtc	atc	1776
Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile	
		580						585					590			
tac	att	aat	aaa	act	atg	aat	aaa	gat	gat	gat	tta	aca	tat	caa	aca	1824
Tyr	Ile	Asn	Lys	Thr	Met	Asn	Lys	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr	
		595				600					605					
ttt	gat	ctc	gca	act	act	aat	tct	aat	atg	ggg	ttc	tcg	ggt	gat	aag	1872
Phe	Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys	
		610				615				620						
aat	gaa	ctt	ata	ata	gga	gca	gaa	tct	ttc	gtt	tct	aat	gaa	aaa	atc	1920
Asn	Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile	
625				630				635						640		
tat	ata	gat	aag	ata	gaa	ttt	atc	cca	gta	caa	ttg	taa				1959
Tyr	Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu					
			645					650								

<210> SEQ ID NO 10

<211> LENGTH: 652

<212> TYPE: PRP

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 10

Met	Asn	Pro	Asn	Asn	Arg	Ser	Glu	His	Asp	Thr	Ile	Lys	Val	Thr	Pro	
1			5						10					15		
Asn	Ser	Glu	Leu	Gln	Thr	Asn	His	Asn	Gln	Tyr	Pro	Leu	Ala	Asp	Asn	
		20					25						30			
Pro	Asn	Ser	Thr	Leu	Glu	Glu	Leu	Asn	Tyr	Lys	Glu	Phe	Leu	Arg	Met	
		35				40					45					
Thr	Glu	Asp	Ser	Ser	Thr	Glu	Val	Leu	Asp	Asn	Ser	Thr	Val	Lys	Asp	
	50				55					60						
Ala	Val	Gly	Thr	Gly	Ile	Ser	Val	Val	Gly	Gln	Ile	Leu	Gly	Val	Val	
65				70					75					80		
Gly	Val	Pro	Phe	Ala	Gly	Ala	Leu	Thr	Ser	Phe	Tyr	Gln	Ser	Phe	Leu	
			85					90						95		
Asn	Thr	Ile	Trp	Pro	Ser	Asp	Ala	Asp	Pro	Trp	Lys	Ala	Phe	Met	Ala	
		100					105					110				
Gln	Val	Glu	Val	Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser	
		115				120						125				
Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr	
	130					135					140					

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Val	Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Thr	Pro	Leu	Ser	Leu	Arg	Ser	145	150	155	160
Lys	Arg	Ser	Gln	Asp	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser	165	170	175	
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val	180	185	190	
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	195	200	205	
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu	210	215	220	
Asp	Val	Ala	Glu	Phe	Tyr	Asn	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr	225	230	235	240
Ser	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg	245	250	255	
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu	260	265	270	
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp	275	280	285	
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile	290	295	300	
Phe	Thr	Asp	Pro	Ile	Phe	Ser	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro	305	310	315	320
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	325	330	335	
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe	340	345	350	
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg	355	360	365	
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp	370	375	380	
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val	385	390	395	400
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys	405	410	415	
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln	420	425	430	
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly	435	440	445	
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	450	455	460	
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu	465	470	475	480
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp	485	490	495	
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile	500	505	510	
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser	515	520	525	
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys	530	535	540	
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala	545	550	555	560

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ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta	624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	
195 200 205	
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa	672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	
210 215 220	
gat gtt gct gaa ttc tat acc aga caa tta aaa ctt aca caa caa tac	720
Asp Val Ala Glu Phe Tyr Thr Arg Gln Leu Lys Leu Thr Gln Gln Tyr	
225 230 235 240	
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga	768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	
245 250 255	
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa	816
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	
260 265 270	
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat	864
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	
275 280 285	
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att	912
Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	
290 295 300	
ttt acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca	960
Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro	
305 310 315 320	
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat	1008
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	
325 330 335	
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt	1056
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe	
340 345 350	
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga	1104
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg	
355 360 365	
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat	1152
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp	
370 375 380	
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt	1200
Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val	
385 390 395 400	
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag	1248
Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys	
405 410 415	
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa	1296
Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln	
420 425 430	
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc	1344
Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly	
435 440 445	
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca	1392
His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr	
450 455 460	
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa	1440
Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu	
465 470 475 480	
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg	1488
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp	
485 490 495	
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att	1536
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile	
500 505 510	

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act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc	1584
Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser	
515 520 525	
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa	1632
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys	
530 535 540	
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca	1680
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala	
545 550 555 560	
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act	1728
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr	
565 570 575	
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc	1776
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile	
580 585 590	
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca	1824
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr	
595 600 605	
ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag	1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys	
610 615 620	
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc	1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile	
625 630 635 640	
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa	1959
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu	
645 650	

<210> SEQ ID NO 12

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 12

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser	
145 150 155 160	
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	
165 170 175	

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His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val	180	185	190	
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	195	200	205	
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu	210	215	220	
Asp	Val	Ala	Glu	Phe	Tyr	Thr	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr	225	230	235	240
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg	245	250	255	
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu	260	265	270	
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp	275	280	285	
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile	290	295	300	
Phe	Thr	Asp	Pro	Ile	Phe	Ser	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro	305	310	315	320
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	325	330	335	
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe	340	345	350	
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg	355	360	365	
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp	370	375	380	
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val	385	390	395	400
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys	405	410	415	
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln	420	425	430	
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly	435	440	445	
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	450	455	460	
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu	465	470	475	480
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp	485	490	495	
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile	500	505	510	
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser	515	520	525	
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys	530	535	540	
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala	545	550	555	560
Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr	565	570	575	
Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile	580	585	590	

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Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
595 600 605

Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
610 615 620

Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
625 630 635 640

Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
645 650

<210> SEQ ID NO 13
<211> LENGTH: 1959
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant delta endotoxin
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1956)

<400> SEQUENCE: 13

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct	48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca	336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt	384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat	432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt	480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser	
145 150 155 160	
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt	528
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	
165 170 175	
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg	576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	
180 185 190	
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta	624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	
195 200 205	
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa	672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	

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210	215	220	
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr 225 230 235 240			720
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg 245 250 255			768
ggg tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu 260 265 270			816
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp 275 280 285			864
att aat tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att Ile Asn Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile 290 295 300			912
ttt acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro 305 310 315 320			960
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp 325 330 335			1008
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe 340 345 350			1056
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg 355 360 365			1104
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp 370 375 380			1152
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 385 390 395 400			1200
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys 405 410 415			1248
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln 420 425 430			1296
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly 435 440 445			1344
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr 450 455 460			1392
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu 465 470 475 480			1440
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp 485 490 495			1488
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile 500 505 510			1536
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser 515 520 525			1584
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa			1632

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Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys	
530 535 540	
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca	1680
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala	
545 550 555 560	
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act	1728
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr	
565 570 575	
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc	1776
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile	
580 585 590	
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca	1824
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr	
595 600 605	
ttt gat ctg gca act act aat tct aat atg ggg ttc tcg ggt gat aag	1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys	
610 615 620	
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc	1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile	
625 630 635 640	
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa	1959
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu	
645 650	

<210> SEQ ID NO 14

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 14

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser	
145 150 155 160	
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	
165 170 175	
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	
180 185 190	
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	
195 200 205	

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Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
 210 215 220
 Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr
 225 230 235 240
 Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg
 245 250 255
 Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu
 260 265 270
 Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp
 275 280 285
 Ile Asn Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile
 290 295 300
 Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro
 305 310 315 320
 Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp
 325 330 335
 Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe
 340 345 350
 Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg
 355 360 365
 Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp
 370 375 380
 Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val
 385 390 395 400
 Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys
 405 410 415
 Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln
 420 425 430
 Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly
 435 440 445
 His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr
 450 455 460
 Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu
 465 470 475 480
 Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp
 485 490 495
 Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile
 500 505 510
 Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser
 515 520 525
 Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys
 530 535 540
 Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
 545 550 555 560
 Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
 565 570 575
 Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
 580 585 590
 Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
 595 600 605
 Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
 610 615 620

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Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
625 630 635 640

Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
645 650

<210> SEQ ID NO 15

<211> LENGTH: 1959

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(1956)

<400> SEQUENCE: 15

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct 48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
1 5 10 15

aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat 96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
20 25 30

cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg 144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
35 40 45

act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat 192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
50 55 60

gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta 240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
65 70 75 80

gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt 288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
85 90 95

aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca 336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
100 105 110

caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt 384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
115 120 125

aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat 432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
130 135 140

gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt 480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
145 150 155 160

aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt 528
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser
165 170 175

cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg 576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val
180 185 190

ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta 624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
195 200 205

tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa 672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
210 215 220

gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac 720
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr
225 230 235 240

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act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga	768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	
245 250 255	
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa	816
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	
260 265 270	
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat	864
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	
275 280 285	
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att	912
Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	
290 295 300	
ttt acg gat cca att ttt tta ctt act acg ctt cag aag tac gga cca	960
Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr Gly Pro	
305 310 315 320	
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat	1008
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	
325 330 335	
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt	1056
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe	
340 345 350	
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga	1104
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg	
355 360 365	
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat	1152
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp	
370 375 380	
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt	1200
Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val	
385 390 395 400	
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag	1248
Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys	
405 410 415	
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa	1296
Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln	
420 425 430	
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc	1344
Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly	
435 440 445	
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca	1392
His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr	
450 455 460	
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa	1440
Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu	
465 470 475 480	
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg	1488
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp	
485 490 495	
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att	1536
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile	
500 505 510	
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc	1584
Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser	
515 520 525	
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa	1632
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys	
530 535 540	
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca	1680
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala	
545 550 555 560	

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gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act 1728
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
      565      570      575

aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc 1776
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
      580      585      590

tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca 1824
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
      595      600      605

ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag 1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
      610      615      620

aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc 1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
      625      630      635      640

tat ata gat aag ata gaa ttt atc cca gta caa ttg taa 1959
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
      645      650

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<210> SEQ ID NO 16
<211> LENGTH: 652
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant delta endotoxin

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<400> SEQUENCE: 16

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Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
1      5      10      15

Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
      20      25      30

Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
      35      40      45

Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
      50      55      60

Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
      65      70      75      80

Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
      85      90      95

Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
      100      105      110

Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
      115      120      125

Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
      130      135      140

Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
      145      150      155      160

Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser
      165      170      175

His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val
      180      185      190

Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
      195      200      205

Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
      210      215      220

Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr
      225      230      235      240

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Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg
				245					250						
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu
				260					265						
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp
				275					280						
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile
				290					295						
Phe	Thr	Asp	Pro	Ile	Phe	Leu	Leu	Thr	Thr	Leu	Gln	Lys	Tyr	Gly	Pro
				305					310						
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp
				325					330						
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe
				340					345						
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg
				355					360						
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp
				370					375						
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val
				385					390						
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys
				405					410						
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln
				420					425						
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly
				435					440						
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr
				450					455						
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu
				465					470						
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp
				485					490						
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile
				500					505						
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser
				515					520						
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys
				530					535						
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala
				545					550						
Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr
				565					570						
Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile
				580					585						
Tyr	Ile	Asn	Lys	Thr	Met	Asn	Lys	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr
				595					600						
Phe	Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys
				610					615						
Asn	Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile
				625					630						
Tyr	Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu				
				645					650						

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<210> SEQ ID NO 17
<211> LENGTH: 1959
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant delta endotoxin
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1956)

<400> SEQUENCE: 17

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct      48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
1          5          10          15

aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat      96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
20          25          30

cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg     144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
35          40          45

act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat     192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
50          55          60

gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta     240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
65          70          75          80

gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt     288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
85          90          95

aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca     336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
100         105         110

caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt     384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
115         120         125

aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat     432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
130         135         140

gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt     480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
145         150         155         160

aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt     528
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser
165         170         175

cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg     576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val
180         185         190

ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta     624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
195         200         205

tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa     672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
210         215         220

gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac     720
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr
225         230         235         240

act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga     768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg
245         250         255

ggg tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa     816
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu

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260	265	270	
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp 275 280 285			864
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile 290 295 300			912
ttt acg gat cca att ttt acc ctt aat aca cta cag aag tgc gga cca Phe Thr Asp Pro Ile Phe Thr Leu Asn Thr Leu Gln Lys Cys Gly Pro 305 310 315 320			960
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp 325 330 335			1008
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe 340 345 350			1056
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg 355 360 365			1104
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp 370 375 380			1152
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 385 390 395 400			1200
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys 405 410 415			1248
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln 420 425 430			1296
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc Lys Asn Gln Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly 435 440 445			1344
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr 450 455 460			1392
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu 465 470 475 480			1440
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp 485 490 495			1488
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile 500 505 510			1536
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser 515 520 525			1584
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys 530 535 540			1632
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala 545 550 555 560			1680
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr 565 570 575			1728
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc			1776

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Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp
 275                280                285

Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile
 290                295                300

Phe Thr Asp Pro Ile Phe Thr Leu Asn Thr Leu Gln Lys Cys Gly Pro
 305                310                315                320

Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp
      325                330                335

Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe
      340                345                350

Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg
 355                360                365

Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp
 370                375                380

Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val
 385                390                395                400

Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys
      405                410                415

Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln
      420                425                430

Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly
 435                440                445

His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr
 450                455                460

Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu
 465                470                475                480

Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp
      485                490                495

Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile
      500                505                510

Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser
      515                520                525

Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys
 530                535                540

Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
 545                550                555                560

Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
      565                570                575

Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
      580                585                590

Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
      595                600                605

Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
      610                615                620

Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
 625                630                635                640

Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
      645                650

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<210> SEQ ID NO 19
<211> LENGTH: 1959
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Recombinant delta endotoxin

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(1956)

<400> SEQUENCE: 19

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct	48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca	336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt	384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat	432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt	480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser	
145 150 155 160	
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt	528
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	
165 170 175	
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg	576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	
180 185 190	
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta	624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	
195 200 205	
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa	672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	
210 215 220	
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac	720
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr	
225 230 235 240	
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga	768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	
245 250 255	
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa	816
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	
260 265 270	
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat	864
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	
275 280 285	

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att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile 290 295 300	912
ttt acg gat cca att ttt gcc gtt aat act ctg tgg gaa tac gga cca Phe Thr Asp Pro Ile Phe Ala Val Asn Thr Leu Trp Glu Tyr Gly Pro 305 310 315 320	960
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp 325 330 335	1008
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe 340 345 350	1056
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg 355 360 365	1104
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp 370 375 380	1152
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 385 390 395 400	1200
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys 405 410 415	1248
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln 420 425 430	1296
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly 435 440 445	1344
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr 450 455 460	1392
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu 465 470 475 480	1440
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp 485 490 495	1488
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile 500 505 510	1536
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser 515 520 525	1584
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys 530 535 540	1632
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala 545 550 555 560	1680
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr 565 570 575	1728
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile 580 585 590	1776
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr 595 600 605	1824

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ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag      1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
    610                      615                      620

aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc      1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
    625                      630                      635                      640

tat ata gat aag ata gaa ttt atc cca gta caa ttg taa      1959
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
    645                      650

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<210> SEQ ID NO 20
<211> LENGTH: 652
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant delta endotoxin

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<400> SEQUENCE: 20

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Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
1                      5                      10                      15

Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
    20                      25                      30

Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
    35                      40                      45

Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
    50                      55                      60

Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
    65                      70                      75                      80

Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
    85                      90                      95

Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
    100                     105                     110

Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
    115                     120                     125

Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
    130                     135                     140

Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
    145                     150                     155                     160

Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser
    165                     170                     175

His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val
    180                     185                     190

Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
    195                     200                     205

Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
    210                     215                     220

Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr
    225                     230                     235                     240

Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg
    245                     250                     255

Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu
    260                     265                     270

Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp
    275                     280                     285

Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile
    290                     295                     300

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Phe Thr Asp Pro Ile Phe Ala Val Asn Thr Leu Trp Glu Tyr Gly Pro
305          310          315          320

Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp
          325          330          335

Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe
          340          345          350

Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg
          355          360          365

Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp
          370          375          380

Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val
385          390          395          400

Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys
          405          410          415

Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln
          420          425          430

Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly
          435          440          445

His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr
          450          455          460

Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu
465          470          475          480

Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp
          485          490          495

Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile
          500          505          510

Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser
          515          520          525

Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys
          530          535          540

Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
545          550          555          560

Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
          565          570          575

Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
          580          585          590

Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
          595          600          605

Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
          610          615          620

Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
625          630          635          640

Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
          645          650

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<210> SEQ ID NO 21
<211> LENGTH: 1959
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant delta endotoxin
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1956)

<400> SEQUENCE: 21

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atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro 1 5 10 15	48
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn 20 25 30	96
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met 35 40 45	144
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp 50 55 60	192
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val 65 70 75 80	240
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu 85 90 95	288
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala 100 105 110	336
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser 115 120 125	384
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr 130 135 140	432
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser 145 150 155 160	480
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser 165 170 175	528
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val 180 185 190	576
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu 195 200 205	624
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu 210 215 220	672
gat gtt gct gaa ttc tat cgt aga caa tta aaa ctt aca caa caa tac Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln Gln Tyr 225 230 235 240	720
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg 245 250 255	768
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu 260 265 270	816
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp 275 280 285	864
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile 290 295 300	912
ttt acg gat cca att ttt tta ctt act acg ctt cag aag tac gga cca Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr Gly Pro	960

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305	310	315	320	
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat				1008
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	325	330	335	
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt				1056
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe	340	345	350	
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga				1104
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg	355	360	365	
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat				1152
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp	370	375	380	
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt				1200
Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val	385	390	395	400
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag				1248
Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys	405	410	415	
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa				1296
Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln	420	425	430	
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc				1344
Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly	435	440	445	
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca				1392
His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr	450	455	460	
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa				1440
Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu	465	470	475	480
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg				1488
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp	485	490	495	
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att				1536
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile	500	505	510	
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc				1584
Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser	515	520	525	
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa				1632
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys	530	535	540	
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca				1680
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala	545	550	555	560
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act				1728
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr	565	570	575	
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc				1776
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile	580	585	590	
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca				1824
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr	595	600	605	
ttt gat ctg gca act act aat tct aat atg ggg ttc tcg ggt gat aag				1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys	610	615	620	
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc				1920

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Asn	Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile	
625					630					635					640	

tat	ata	gat	aag	ata	gaa	ttt	atc	cca	gta	caa	ttg	taa		1959
Tyr	Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu			
				645					650					

<210> SEQ ID NO 22
 <211> LENGTH: 652
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 22

Met	Asn	Pro	Asn	Asn	Arg	Ser	Glu	His	Asp	Thr	Ile	Lys	Val	Thr	Pro	
1			5						10					15		

Asn	Ser	Glu	Leu	Gln	Thr	Asn	His	Asn	Gln	Tyr	Pro	Leu	Ala	Asp	Asn	
			20					25					30			

Pro	Asn	Ser	Thr	Leu	Glu	Glu	Leu	Asn	Tyr	Lys	Glu	Phe	Leu	Arg	Met	
		35					40					45				

Thr	Glu	Asp	Ser	Ser	Thr	Glu	Val	Leu	Asp	Asn	Ser	Thr	Val	Lys	Asp	
	50					55					60					

Ala	Val	Gly	Thr	Gly	Ile	Ser	Val	Val	Gly	Gln	Ile	Leu	Gly	Val	Val	
65					70					75					80	

Gly	Val	Pro	Phe	Ala	Gly	Ala	Leu	Thr	Ser	Phe	Tyr	Gln	Ser	Phe	Leu	
				85					90					95		

Asn	Thr	Ile	Trp	Pro	Ser	Asp	Ala	Asp	Pro	Trp	Lys	Ala	Phe	Met	Ala	
			100					105					110			

Gln	Val	Glu	Val	Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser	
		115					120					125				

Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr	
	130					135						140				

Val	Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Thr	Pro	Leu	Ser	Leu	Arg	Ser	
145					150					155					160	

Lys	Arg	Ser	Gln	Asp	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser	
				165					170					175		

His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val	
			180					185					190			

Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	
		195					200					205				

Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu	
	210						215					220				

Asp	Val	Ala	Glu	Phe	Tyr	Arg	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr	
225					230					235					240	

Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg	
				245					250					255		

Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu	
			260					265					270			

Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp	
		275					280					285				

Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile	
		290					295					300				

Phe	Thr	Asp	Pro	Ile	Phe	Leu	Leu	Thr	Thr	Leu	Gln	Lys	Tyr	Gly	Pro	
305					310					315					320	

Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	
				325					330					335		

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Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe
 340 345 350
 Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg
 355 360 365
 Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp
 370 375 380
 Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val
 385 390 395 400
 Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys
 405 410 415
 Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln
 420 425 430
 Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly
 435 440 445
 His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr
 450 455 460
 Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu
 465 470 475 480
 Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp
 485 490 495
 Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile
 500 505 510
 Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser
 515 520 525
 Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys
 530 535 540
 Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
 545 550 555 560
 Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
 565 570 575
 Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
 580 585 590
 Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
 595 600 605
 Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
 610 615 620
 Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
 625 630 635 640
 Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> SEQ ID NO 23
 <211> LENGTH: 1959
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant delta endotoxin
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1956)

<400> SEQUENCE: 23

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct 48
 Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
 1 5 10 15
 aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat 96

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Asn	Ser	Glu	Leu	Gln	Thr	Asn	His	Asn	Gln	Tyr	Pro	Leu	Ala	Asp	Asn	
			20					25					30			
cca	aat	tca	aca	cta	gaa	gaa	tta	aat	tat	aaa	gaa	ttt	tta	aga	atg	144
Pro	Asn	Ser	Thr	Leu	Glu	Glu	Leu	Asn	Tyr	Lys	Glu	Phe	Leu	Arg	Met	
	35						40					45				
act	gaa	gac	agt	tct	acg	gaa	gtg	cta	gac	aac	tct	aca	gta	aaa	gat	192
Thr	Glu	Asp	Ser	Ser	Thr	Glu	Val	Leu	Asp	Asn	Ser	Thr	Val	Lys	Asp	
	50					55				60						
gca	gtt	ggg	aca	gga	att	tct	gtt	gta	ggg	cag	att	tta	ggt	gtt	gta	240
Ala	Val	Gly	Thr	Gly	Ile	Ser	Val	Val	Gly	Gln	Ile	Leu	Gly	Val	Val	
65					70				75					80		
gga	gtt	cca	ttt	gct	ggg	gca	ctc	act	tca	ttt	tat	caa	tca	ttt	ctt	288
Gly	Val	Pro	Phe	Ala	Gly	Ala	Leu	Thr	Ser	Phe	Tyr	Gln	Ser	Phe	Leu	
			85					90						95		
aac	act	ata	tgg	cca	agt	gat	gct	gac	cca	tgg	aag	gct	ttt	atg	gca	336
Asn	Thr	Ile	Trp	Pro	Ser	Asp	Ala	Asp	Pro	Trp	Lys	Ala	Phe	Met	Ala	
		100						105					110			
caa	gtt	gaa	gta	ctg	ata	gat	aag	aaa	ata	gag	gag	tat	gct	aaa	agt	384
Gln	Val	Glu	Val	Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser	
	115						120					125				
aaa	gct	ctt	gca	gag	tta	cag	ggg	ctt	caa	aat	aat	ttc	gaa	gat	tat	432
Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr	
	130					135				140						
gtt	aat	gcg	tta	aat	tcc	tgg	aag	aaa	aca	cct	tta	agt	ttg	cga	agt	480
Val	Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Thr	Pro	Leu	Ser	Leu	Arg	Ser	
145					150					155					160	
aaa	aga	agc	caa	gat	cga	ata	agg	gaa	ctt	ttt	tct	caa	gca	gaa	agt	528
Lys	Arg	Ser	Gln	Asp	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser	
			165					170						175		
cat	ttt	cgt	aat	tcc	atg	ccg	tca	ttt	gca	gtt	tcc	aaa	ttc	gaa	gtg	576
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val	
		180						185					190			
ctg	ttt	cta	cca	aca	tat	gca	caa	gct	gca	aat	aca	cat	tta	ttg	cta	624
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	
		195					200					205				
tta	aaa	gat	gct	caa	gtt	ttt	gga	gaa	gaa	tgg	gga	tat	tct	tca	gaa	672
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu	
	210						215				220					
gat	gtt	gct	gaa	ttt	tat	cat	aga	caa	tta	aaa	ctt	aca	caa	caa	tac	720
Asp	Val	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr	
225					230					235					240	
act	gac	cat	tgt	gtt	aat	tgg	tat	aat	gtt	gga	tta	aat	ggt	tta	aga	768
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg	
			245						250					255		
ggt	tca	act	tat	gat	gca	tgg	gtc	aaa	ttt	aac	cgt	ttt	cgc	aga	gaa	816
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu	
		260						265					270			
atg	act	tta	act	gta	tta	gat	cta	att	gta	ctt	ttc	cca	ttt	tat	gat	864
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp	
		275					280					285				
att	cgg	tta	tac	tca	aaa	ggg	gtt	aaa	aca	gaa	cta	aca	aga	gac	att	912
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile	
		290					295					300				
ttt	acg	gat	cca	att	ttt	acg	cca	acc	acc	cta	cag	gat	tac	gga	cca	960
Phe	Thr	Asp	Pro	Ile	Phe	Thr	Pro	Thr	Thr	Leu	Gln	Asp	Tyr	Gly	Pro	
305					310					315					320	
act	ttt	ttg	agt	ata	gaa	aac	tct	att	cga	aaa	cct	cat	tta	ttt	gat	1008
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	
				325					330					335		

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tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt	1056
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe	
340 345 350	
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga	1104
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg	
355 360 365	
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat	1152
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp	
370 375 380	
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt	1200
Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val	
385 390 395 400	
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag	1248
Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys	
405 410 415	
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa	1296
Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln	
420 425 430	
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc	1344
Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly	
435 440 445	
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca	1392
His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr	
450 455 460	
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa	1440
Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu	
465 470 475 480	
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg	1488
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp	
485 490 495	
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att	1536
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile	
500 505 510	
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc	1584
Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser	
515 520 525	
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa	1632
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys	
530 535 540	
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca	1680
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala	
545 550 555 560	
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act	1728
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr	
565 570 575	
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc	1776
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile	
580 585 590	
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca	1824
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr	
595 600 605	
ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag	1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys	
610 615 620	
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc	1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile	
625 630 635 640	
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa	1959
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu	
645 650	

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<210> SEQ ID NO 24
<211> LENGTH: 652
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 24

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
1          5          10          15

Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
20          25          30

Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
35          40          45

Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
50          55          60

Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
65          70          75          80

Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
85          90          95

Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
100         105         110

Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
115         120         125

Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
130         135         140

Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
145         150         155         160

Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser
165         170         175

His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val
180         185         190

Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
195         200         205

Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
210         215         220

Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr
225         230         235         240

Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg
245         250         255

Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu
260         265         270

Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp
275         280         285

Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile
290         295         300

Phe Thr Asp Pro Ile Phe Thr Pro Thr Thr Leu Gln Asp Tyr Gly Pro
305         310         315         320

Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp
325         330         335

Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe
340         345         350

Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg
355         360         365

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Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp
 370 375 380
 Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val
 385 390 395 400
 Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys
 405 410 415
 Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln
 420 425 430
 Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly
 435 440 445
 His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr
 450 455 460
 Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu
 465 470 475 480
 Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp
 485 490 495
 Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile
 500 505 510
 Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser
 515 520 525
 Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Phe Leu Lys
 530 535 540
 Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
 545 550 555 560
 Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
 565 570 575
 Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
 580 585 590
 Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
 595 600 605
 Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
 610 615 620
 Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
 625 630 635 640
 Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> SEQ ID NO 25

<211> LENGTH: 1959

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(1956)

<400> SEQUENCE: 25

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct	48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	

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act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp 50 55 60	192
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val 65 70 75 80	240
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu 85 90 95	288
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala 100 105 110	336
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser 115 120 125	384
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr 130 135 140	432
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser 145 150 155 160	480
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser 165 170 175	528
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val 180 185 190	576
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu 195 200 205	624
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu 210 215 220	672
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr 225 230 235 240	720
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg 245 250 255	768
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu 260 265 270	816
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp 275 280 285	864
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile 290 295 300	912
ttt acg gat cca att ttt gcc ctg aat acc tta gac gag tac gga cca Phe Thr Asp Pro Ile Phe Ala Leu Asn Thr Leu Asp Glu Tyr Gly Pro 305 310 315 320	960
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp 325 330 335	1008
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe 340 345 350	1056
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg	1104

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355	360	365	
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp 370 375 380			1152
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 385 390 395 400			1200
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys 405 410 415			1248
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln 420 425 430			1296
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly 435 440 445			1344
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr 450 455 460			1392
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu 465 470 475 480			1440
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp 485 490 495			1488
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile 500 505 510			1536
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser 515 520 525			1584
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys 530 535 540			1632
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala 545 550 555 560			1680
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr 565 570 575			1728
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile 580 585 590			1776
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr 595 600 605			1824
ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys 610 615 620			1872
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile 625 630 635 640			1920
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu 645 650			1959

<210> SEQ ID NO 26

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

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<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 26

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Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
1          5          10          15

Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
20          25          30

Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
35          40          45

Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
50          55          60

Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
65          70          75          80

Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
85          90          95

Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
100         105         110

Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
115         120         125

Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
130         135         140

Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
145         150         155         160

Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser
165         170         175

His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val
180         185         190

Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
195         200         205

Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
210         215         220

Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr
225         230         235         240

Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg
245         250         255

Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu
260         265         270

Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp
275         280         285

Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile
290         295         300

Phe Thr Asp Pro Ile Phe Ala Leu Asn Thr Leu Asp Glu Tyr Gly Pro
305         310         315         320

Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp
325         330         335

Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe
340         345         350

Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg
355         360         365

Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp
370         375         380

Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val
385         390         395         400

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Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys
 405 410 415
 Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln
 420 425 430
 Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly
 435 440 445
 His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr
 450 455 460
 Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu
 465 470 475 480
 Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp
 485 490 495
 Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile
 500 505 510
 Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser
 515 520 525
 Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Phe Leu Lys
 530 535 540
 Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
 545 550 555 560
 Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
 565 570 575
 Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
 580 585 590
 Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
 595 600 605
 Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
 610 615 620
 Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
 625 630 635 640
 Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> SEQ ID NO 27

<211> LENGTH: 1959

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(1956)

<400> SEQUENCE: 27

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct	48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240

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Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca	336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt	384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat	432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt	480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser	
145 150 155 160	
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt	528
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	
165 170 175	
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg	576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	
180 185 190	
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta	624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	
195 200 205	
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa	672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	
210 215 220	
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac	720
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr	
225 230 235 240	
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga	768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	
245 250 255	
ggg tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa	816
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	
260 265 270	
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tac gat	864
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	
275 280 285	
act agg cga ttc aga aag ggg gtt aaa aca gaa cta aca aga gac att	912
Thr Arg Arg Phe Arg Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	
290 295 300	
ttt acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca	960
Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro	
305 310 315 320	
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat	1008
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	
325 330 335	
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt	1056
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe	
340 345 350	
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga	1104
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg	
355 360 365	
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat	1152
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp	
370 375 380	

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aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 385 390 395 400	1200
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys 405 410 415	1248
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln 420 425 430	1296
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly 435 440 445	1344
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr 450 455 460	1392
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu 465 470 475 480	1440
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp 485 490 495	1488
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile 500 505 510	1536
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser 515 520 525	1584
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys 530 535 540	1632
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala 545 550 555 560	1680
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr 565 570 575	1728
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile 580 585 590	1776
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr 595 600 605	1824
ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys 610 615 620	1872
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile 625 630 635 640	1920
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu 645 650	1959

<210> SEQ ID NO 28

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 28

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro

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1	5	10	15
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	20	25	30
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	35	40	45
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	50	55	60
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	65	70	75
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	85	90	95
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	100	105	110
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	115	120	125
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	130	135	140
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser	145	150	155
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	165	170	175
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	180	185	190
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	195	200	205
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	210	215	220
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr	225	230	235
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	245	250	255
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	260	265	270
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	275	280	285
Thr Arg Arg Phe Arg Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	290	295	300
Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro	305	310	315
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	325	330	335
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe	340	345	350
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg	355	360	365
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp	370	375	380
Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val	385	390	395
Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys	405	410	415
Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln	420	425	430

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Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly
 435 440 445
 His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Glu Thr Thr
 450 455 460
 Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu
 465 470 475 480
 Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp
 485 490 495
 Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile
 500 505 510
 Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser
 515 520 525
 Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys
 530 535 540
 Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
 545 550 555 560
 Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
 565 570 575
 Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
 580 585 590
 Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
 595 600 605
 Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
 610 615 620
 Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
 625 630 635 640
 Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> SEQ ID NO 29
 <211> LENGTH: 1959
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant delta endotoxin
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1956)

<400> SEQUENCE: 29

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct	48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	

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aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala 100 105 110	336
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser 115 120 125	384
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr 130 135 140	432
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser 145 150 155 160	480
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser 165 170 175	528
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val 180 185 190	576
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu 195 200 205	624
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu 210 215 220	672
gat gtt gct gaa ttc tat cgt aga caa tta aaa ctt aca caa caa tac Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln Gln Tyr 225 230 235 240	720
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg 245 250 255	768
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu 260 265 270	816
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp 275 280 285	864
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile 290 295 300	912
ttt acg gat cca att ttt tta ctt aat act ctt cag gag tat gga cca Phe Thr Asp Pro Ile Phe Leu Leu Asn Thr Leu Gln Glu Tyr Gly Pro 305 310 315 320	960
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp 325 330 335	1008
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe 340 345 350	1056
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg 355 360 365	1104
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp 370 375 380	1152
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 385 390 395 400	1200
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys	1248

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405	410	415	
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln 420 425 430			1296
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly 435 440 445			1344
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr 450 455 460			1392
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu 465 470 475 480			1440
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp 485 490 495			1488
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile 500 505 510			1536
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser 515 520 525			1584
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys 530 535 540			1632
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala 545 550 555 560			1680
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr 565 570 575			1728
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile 580 585 590			1776
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr 595 600 605			1824
ttt gat ctg gca act act aat tct aat atg ggg ttc tcg ggt gat aag Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys 610 615 620			1872
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile 625 630 635 640			1920
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu 645 650			1959

<210> SEQ ID NO 30

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 30

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
1 5 10 15

Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
20 25 30

Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met

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35					40					45					
Thr	Glu	Asp	Ser	Ser	Thr	Glu	Val	Leu	Asp	Asn	Ser	Thr	Val	Lys	Asp
	50					55					60				
Ala	Val	Gly	Thr	Gly	Ile	Ser	Val	Val	Gly	Gln	Ile	Leu	Gly	Val	Val
65					70					75					80
Gly	Val	Pro	Phe	Ala	Gly	Ala	Leu	Thr	Ser	Phe	Tyr	Gln	Ser	Phe	Leu
				85					90					95	
Asn	Thr	Ile	Trp	Pro	Ser	Asp	Ala	Asp	Pro	Trp	Lys	Ala	Phe	Met	Ala
			100					105					110		
Gln	Val	Glu	Val	Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser
		115					120					125			
Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr
	130					135					140				
Val	Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Thr	Pro	Leu	Ser	Leu	Arg	Ser
145					150					155					160
Lys	Arg	Ser	Gln	Asp	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser
				165					170					175	
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val
			180					185					190		
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu
		195					200					205			
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu
	210					215					220				
Asp	Val	Ala	Glu	Phe	Tyr	Arg	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr
225					230					235					240
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg
				245					250					255	
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu
			260					265					270		
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp
		275					280					285			
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile
	290					295						300			
Phe	Thr	Asp	Pro	Ile	Phe	Leu	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro
305					310					315					320
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp
				325					330					335	
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe
			340					345					350		
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg
		355					360					365			
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp
	370					375					380				
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val
385					390					395					400
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys
				405					410					415	
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln
			420					425					430		
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly
		435					440					445			
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr
	450					455					460				

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Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu
 465 470 475 480
 Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp
 485 490 495
 Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile
 500 505 510
 Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser
 515 520 525
 Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Phe Leu Lys
 530 535 540
 Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
 545 550 555 560
 Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
 565 570 575
 Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
 580 585 590
 Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
 595 600 605
 Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
 610 615 620
 Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
 625 630 635 640
 Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> SEQ ID NO 31
 <211> LENGTH: 1959
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant delta endotoxin
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1956)

<400> SEQUENCE: 31

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct	48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca	336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt	384

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Gln	Val	Glu	Val	Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser	
	115						120					125				
aaa	gct	ctt	gca	gag	tta	cag	ggt	ctt	caa	aat	aat	ttc	gaa	gat	tat	432
Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr	
	130					135				140						
ggt	aat	gcg	tta	aat	tcc	tggt	aag	aaa	aca	cct	tta	agt	ttg	cga	agt	480
Val	Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Thr	Pro	Leu	Ser	Leu	Arg	Ser	
	145				150					155					160	
aaa	aga	agc	caa	gat	cga	ata	agg	gaa	ctt	ttt	tct	caa	gca	gaa	agt	528
Lys	Arg	Ser	Gln	Asp	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser	
			165					170						175		
cat	ttt	cg	aat	tcc	atg	ccg	tca	ttt	gca	gtt	tcc	aaa	ttc	gaa	gtg	576
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val	
			180					185						190		
ctg	ttt	cta	cca	aca	tat	gca	caa	gct	gca	aat	aca	cat	tta	ttg	cta	624
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	
		195				200						205				
tta	aaa	gat	gct	caa	ggt	ttt	gga	gaa	gaa	tggt	gga	tat	tct	tca	gaa	672
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu	
	210					215					220					
gat	gtt	gct	gaa	ttt	tat	cat	aga	caa	tta	aaa	ctt	aca	caa	caa	tac	720
Asp	Val	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr	
	225				230					235					240	
act	gac	cat	tgt	gtt	aat	tggt	tat	aat	gtt	gga	tta	aat	ggt	tta	aga	768
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg	
			245					250						255		
ggt	tca	act	tat	gat	gca	tggt	gtc	aaa	ttt	aac	cg	ttt	cg	aga	gaa	816
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu	
		260					265						270			
atg	act	tta	act	gta	tta	gat	cta	att	gta	ctt	ttc	cca	ttt	tat	gat	864
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp	
		275				280						285				
att	cg	tta	tac	tca	aaa	gggt	gtt	aaa	aca	gaa	cta	aca	aga	gac	att	912
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile	
	290					295					300					
ttt	acg	gat	cca	att	ttt	atc	ctc	aat	acg	cta	cag	gag	tac	gga	cca	960
Phe	Thr	Asp	Pro	Ile	Phe	Ile	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro	
	305				310					315					320	
act	ttt	ttg	agt	ata	gaa	aac	tct	att	cga	aaa	cct	cat	tta	ttt	gat	1008
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	
		325						330						335		
tat	tta	cag	ggg	att	gaa	ttt	cat	acg	cg	ctt	caa	cct	ggt	tac	ttt	1056
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe	
		340						345					350			
ggg	aaa	gat	tct	ttc	aat	tat	tggt	tct	ggt	aat	tat	gta	gaa	act	aga	1104
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg	
	355						360					365				
cct	agt	ata	gga	tct	agt	aag	aca	att	act	tcc	cca	ttt	tat	gga	gat	1152
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp	
	370					375					380					
aaa	tct	act	gaa	cct	gta	caa	aag	cta	agc	ttt	gat	gga	caa	aaa	gtt	1200
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val	
	385				390					395					400	
tat	cga	act	ata	gct	aat	aca	gac	gta	gcg	gct	tggt	ccg	aat	ggt	aag	1248
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys	
			405					410						415		
gta	tat	tta	ggt	gtt	acg	aaa	gtt	gat	ttt	agt	caa	tat	gat	gat	caa	1296
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln	
		420						425					430			

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aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc	1344
Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly	
435 440 445	
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca	1392
His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr	
450 455 460	
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa	1440
Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu	
465 470 475 480	
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg	1488
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp	
485 490 495	
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att	1536
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile	
500 505 510	
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc	1584
Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser	
515 520 525	
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa	1632
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys	
530 535 540	
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca	1680
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala	
545 550 555 560	
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act	1728
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr	
565 570 575	
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc	1776
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile	
580 585 590	
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca	1824
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr	
595 600 605	
ttt gat ctg gca act act aat tct aat atg ggg ttc tcg ggt gat aag	1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys	
610 615 620	
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc	1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile	
625 630 635 640	
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa	1959
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu	
645 650	

<210> SEQ ID NO 32

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 32

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	

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65	70	75	80
Gly Val Pro Phe Ala 85	Gly Ala Leu Thr Ser 90	Phe Tyr Gln Ser Phe Leu 95	
Asn Thr Ile Trp 100	Pro Ser Asp Ala Asp 105	Pro Trp Lys Ala Phe Met Ala 110	
Gln Val Glu Val Leu Ile 115	Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser 120		
Lys Ala Leu Ala Glu Leu 130	Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr 135		
Val Asn Ala Leu Asn Ser 145	Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser 150		
Lys Arg Ser Gln Asp 165	Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser 170		
His Phe Arg Asn Ser Met 180	Pro Ser Phe Ala Val Ser Lys Phe Glu Val 185		
Leu Phe Leu Pro Thr Tyr 195	Ala Gln Ala Ala Asn Thr His Leu Leu Leu 200		
Leu Lys Asp Ala Gln Val 210	Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu 215		
Asp Val Ala Glu Phe Tyr 225	His Arg Gln Leu Lys Leu Thr Gln Gln Tyr 230		
Thr Asp His Cys Val 245	Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg 250		
Gly Ser Thr Tyr Asp 260	Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu 265		
Met Thr Leu Thr Val Leu 275	Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp 280		
Ile Arg Leu Tyr Ser Lys 290	Gly Val Lys Thr Glu Leu Thr Arg Asp Ile 295		
Phe Thr Asp Pro Ile Phe 305	Ile Leu Asn Thr Leu Gln Glu Tyr Gly Pro 310		
Thr Phe Leu Ser Ile Glu 325	Asn Ser Ile Arg Lys Pro His Leu Phe Asp 330		
Tyr Leu Gln Gly Ile Glu 340	Phe His Thr Arg Leu Gln Pro Gly Tyr Phe 345		
Gly Lys Asp Ser Phe Asn 355	Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg 360		
Pro Ser Ile Gly Ser Ser 370	Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp 375		
Lys Ser Thr Glu Pro Val 385	Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 390		
Tyr Arg Thr Ile Ala Asn 405	Thr Asp Val Ala Ala Trp Pro Asn Gly Lys 410		
Val Tyr Leu Gly Val Thr 420	Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln 425		
Lys Asn Glu Thr Ser Thr 435	Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly 440		
His Val Ser Ala Gln Asp 450	Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr 455		
Asp Glu Pro Leu Glu Lys 465	Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu 470		
Cys Phe Leu Met Gln Asp 485	Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp 490		

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Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile
 500 505 510

Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser
 515 520 525

Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Phe Leu Lys
 530 535 540

Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
 545 550 555 560

Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
 565 570 575

Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
 580 585 590

Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
 595 600 605

Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
 610 615 620

Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
 625 630 635 640

Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> SEQ ID NO 33

<211> LENGTH: 1959

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(1956)

<400> SEQUENCE: 33

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct	48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca	336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt	384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat	432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	

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gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser 145 150 155 160	480
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser 165 170 175	528
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val 180 185 190	576
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu 195 200 205	624
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu 210 215 220	672
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr 225 230 235 240	720
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg 245 250 255	768
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu 260 265 270	816
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp 275 280 285	864
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile 290 295 300	912
ttt acg gat cca att ttt atc cta cat acg ctg cag gag tac gga cca Phe Thr Asp Pro Ile Phe Ile Leu His Thr Leu Gln Glu Tyr Gly Pro 305 310 315 320	960
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp 325 330 335	1008
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe 340 345 350	1056
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg 355 360 365	1104
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp 370 375 380	1152
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 385 390 395 400	1200
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys 405 410 415	1248
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln 420 425 430	1296
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly 435 440 445	1344
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr	1392

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450	455	460	
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu 465 470 475 480			1440
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp 485 490 495			1488
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile 500 505 510			1536
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser 515 520 525			1584
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys 530 535 540			1632
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala 545 550 555 560			1680
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr 565 570 575			1728
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile 580 585 590			1776
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr 595 600 605			1824
ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys 610 615 620			1872
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile 625 630 635 640			1920
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu 645 650			1959

<210> SEQ ID NO 34

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 34

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
1 5 10 15

Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
20 25 30

Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
35 40 45

Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
50 55 60

Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
65 70 75 80

Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
85 90 95

Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala

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100							105					110				
Gln	Val	Glu	Val	Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser	
		115					120					125				
Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr	
	130					135					140					
Val	Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Thr	Pro	Leu	Ser	Leu	Arg	Ser	
145					150					155					160	
Lys	Arg	Ser	Gln	Asp	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser	
				165					170					175		
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val	
			180					185					190			
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	
		195					200					205				
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu	
	210					215					220					
Asp	Val	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr	
225					230					235					240	
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg	
				245					250					255		
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu	
			260					265					270			
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp	
		275					280					285				
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile	
	290					295					300					
Phe	Thr	Asp	Pro	Ile	Phe	Ile	Leu	His	Thr	Leu	Gln	Glu	Tyr	Gly	Pro	
305					310					315					320	
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	
				325					330					335		
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe	
			340					345					350			
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg	
		355					360					365				
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp	
	370					375					380					
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val	
385					390					395					400	
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys	
				405					410					415		
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln	
			420					425					430			
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly	
		435					440					445				
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	
					455						460					
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu	
465					470					475					480	
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp	
				485					490					495		
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile	
			500					505					510			
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser	
		515					520					525				

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Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys
 530                      535                      540

Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
545                      550                      555                      560

Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
                    565                      570                      575

Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
                    580                      585                      590

Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
                    595                      600                      605

Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
610                      615                      620

Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
625                      630                      635                      640

Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
                    645                      650

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<210> SEQ ID NO 35
<211> LENGTH: 1959
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant delta endotoxin
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1956)

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<400> SEQUENCE: 35

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atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct      48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
 1                      5                      10                      15

aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat      96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
20                      25                      30

cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg     144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
35                      40                      45

act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat     192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
50                      55                      60

gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta     240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
65                      70                      75                      80

gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt     288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
85                      90                      95

aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca     336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
100                     105                     110

caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt     384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
115                     120                     125

aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat     432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
130                     135                     140

gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt     480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
145                     150                     155                     160

aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt     528

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Lys	Arg	Ser	Gln	Asp	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser		
			165						170					175			
cat	ttt	cgt	aat	tcc	atg	ccg	tca	ttt	gca	gtt	tcc	aaa	ttc	gaa	gtg	576	
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val		
			180					185					190				
ctg	ttt	cta	cca	aca	tat	gca	caa	gct	gca	aat	aca	cat	tta	ttg	cta	624	
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu		
		195				200					205						
tta	aaa	gat	gct	caa	gtt	ttt	gga	gaa	gaa	tggt	gga	tat	tct	tca	gaa	672	
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu		
	210					215					220						
gat	gtt	gct	gaa	ttt	tat	cat	aga	caa	tta	aaa	ctt	aca	caa	caa	tac	720	
Asp	Val	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr		
	225				230					235					240		
act	gac	cat	tgt	gtt	aat	tggt	tat	aat	gtt	gga	tta	aat	ggt	tta	aga	768	
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg		
			245					250						255			
ggt	tca	act	tat	gat	gca	tggt	gtc	aaa	ttt	aac	cgt	ttt	cgc	aga	gaa	816	
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu		
		260					265						270				
atg	act	tta	act	gta	tta	gat	cta	att	gta	ctt	ttc	cca	ttt	tat	gat	864	
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp		
		275				280						285					
att	cgt	tta	tac	tca	aaa	gggt	gtt	aaa	aca	gaa	cta	aca	aga	gac	att	912	
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile		
	290					295					300						
ttt	acg	gat	cca	att	ttt	tcc	ctc	gtt	aac	cta	atg	gtg	tac	gga	cca	960	
Phe	Thr	Asp	Pro	Ile	Phe	Ser	Leu	Val	Asn	Leu	Met	Val	Tyr	Gly	Pro		
	305				310				315					320			
act	ttt	ttg	agt	ata	gaa	aac	tct	att	cga	aaa	cct	cat	tta	ttt	gat	1008	
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp		
			325					330						335			
tat	tta	cag	ggg	att	gaa	ttt	cat	acg	cgt	ctt	caa	cct	ggt	tac	ttt	1056	
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe		
		340						345					350				
ggg	aaa	gat	tct	ttc	aat	tat	tggt	tct	ggt	aat	tat	gta	gaa	act	aga	1104	
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg		
		355					360					365					
cct	agt	ata	gga	tct	agt	aag	aca	att	act	tcc	cca	ttt	tat	gga	gat	1152	
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp		
	370					375					380						
aaa	tct	act	gaa	cct	gta	caa	aag	cta	agc	ttt	gat	gga	caa	aaa	gtt	1200	
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val		
	385				390					395				400			
tat	cga	act	ata	gct	aat	aca	gac	gta	gcg	gct	tggt	ccg	aat	ggt	aag	1248	
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys		
			405					410						415			
gta	tat	tta	ggt	gtt	acg	aaa	gtt	gat	ttt	agt	caa	tat	gat	gat	caa	1296	
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln		
		420					425						430				
aaa	aat	gaa	act	agt	aca	caa	aca	tat	gat	tca	aaa	aga	aac	aat	ggc	1344	
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly		
		435				440						445					
cat	gta	agt	gca	cag	gat	tct	att	gac	caa	tta	ccg	cca	gaa	aca	aca	1392	
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr		
		450				455					460						
gat	gaa	cca	ctt	gaa	aaa	gca	tat	agt	cat	cag	ctt	aat	tac	gcg	gaa	1440	
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu		
	465				470					475					480		

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tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg	1488
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp	
485 490 495	
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att	1536
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile	
500 505 510	
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc	1584
Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser	
515 520 525	
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa	1632
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys	
530 535 540	
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca	1680
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala	
545 550 555 560	
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act	1728
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr	
565 570 575	
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc	1776
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile	
580 585 590	
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca	1824
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr	
595 600 605	
ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag	1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys	
610 615 620	
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc	1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile	
625 630 635 640	
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa	1959
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu	
645 650	

<210> SEQ ID NO 36

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 36

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	

130					135					140					
Val 145	Asn	Ala	Leu	Asn 150	Ser	Trp	Lys	Lys	Thr	Pro 155	Leu	Ser	Leu	Arg	Ser 160
Lys	Arg	Ser	Gln 165	Asp	Arg	Ile	Arg	Glu	Leu 170	Phe	Ser	Gln	Ala	Glu	Ser 175
His	Phe	Arg	Asn 180	Ser	Met	Pro	Ser	Phe 185	Ala	Val	Ser	Lys	Phe	Glu	Val 190
Leu	Phe	Leu 195	Pro	Thr	Tyr	Ala	Gln 200	Ala	Ala	Asn	Thr	His 205	Leu	Leu	Leu
Leu	Lys 210	Asp	Ala	Gln	Val 215	Phe	Gly	Glu	Glu	Trp	Gly 220	Tyr	Ser	Ser	Glu
Asp 225	Val	Ala	Glu	Phe	Tyr 230	His	Arg	Gln	Leu	Lys 235	Leu	Thr	Gln	Gln	Tyr 240
Thr	Asp	His	Cys 245	Val	Asn	Trp	Tyr	Asn	Val 250	Gly	Leu	Asn	Gly	Leu	Arg 255
Gly	Ser	Thr	Tyr 260	Asp	Ala	Trp	Val	Lys 265	Phe	Asn	Arg	Phe	Arg 270	Arg	Glu
Met	Thr	Leu 275	Thr	Val	Leu	Asp	Leu 280	Ile	Val	Leu	Phe	Pro 285	Phe	Tyr	Asp
Ile	Arg 290	Leu	Tyr	Ser	Lys	Gly 295	Val	Lys	Thr	Glu	Leu 300	Thr	Arg	Asp	Ile
Phe 305	Thr	Asp	Pro	Ile	Phe 310	Ser	Leu	Val	Asn	Leu 315	Met	Val	Tyr	Gly	Pro 320
Thr	Phe	Leu	Ser	Ile 325	Glu	Asn	Ser	Ile	Arg 330	Lys	Pro	His	Leu	Phe	Asp 335
Tyr	Leu	Gln	Gly 340	Ile	Glu	Phe	His	Thr 345	Arg	Leu	Gln	Pro	Gly	Tyr	Phe 350
Gly	Lys	Asp 355	Ser	Phe	Asn	Tyr	Trp 360	Ser	Gly	Asn	Tyr	Val 365	Glu	Thr	Arg
Pro	Ser 370	Ile	Gly	Ser	Ser	Lys 375	Thr	Ile	Thr	Ser	Pro 380	Phe	Tyr	Gly	Asp
Lys 385	Ser	Thr	Glu	Pro	Val 390	Gln	Lys	Leu	Ser	Phe 395	Asp	Gly	Gln	Lys	Val 400
Tyr	Arg	Thr	Ile	Ala 405	Asn	Thr	Asp	Val	Ala 410	Ala	Trp	Pro	Asn	Gly	Lys 415
Val	Tyr	Leu	Gly 420	Val	Thr	Lys	Val	Asp 425	Phe	Ser	Gln	Tyr	Asp 430	Asp	Gln
Lys	Asn	Glu 435	Thr	Ser	Thr	Gln	Thr 440	Tyr	Asp	Ser	Lys	Arg 445	Asn	Asn	Gly
His	Val 450	Ser	Ala	Gln	Asp	Ser	Ile 455	Asp	Gln	Leu	Pro 460	Pro	Glu	Thr	Thr
Asp 465	Glu	Pro	Leu	Glu	Lys 470	Ala	Tyr	Ser	His	Gln 475	Leu	Asn	Tyr	Ala	Glu 480
Cys	Phe	Leu	Met	Gln 485	Asp	Arg	Arg	Gly	Thr 490	Ile	Pro	Phe	Phe	Thr	Trp 495
Thr	His	Arg	Ser 500	Val	Asp	Phe	Phe	Asn 505	Thr	Ile	Asp	Ala	Glu 510	Lys	Ile
Thr	Gln	Leu 515	Pro	Val	Val	Lys	Ala 520	Tyr	Ala	Leu	Ser	Ser 525	Gly	Ala	Ser
Ile	Ile 530	Glu	Gly	Pro	Gly 535	Phe	Thr	Gly	Gly	Asn	Leu 540	Leu	Phe	Leu	Lys
Glu 545	Ser	Ser	Asn	Ser	Ile 550	Ala	Lys	Phe	Lys	Val 555	Thr	Leu	Asn	Ser	Ala 560

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Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
565 570 575

Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
580 585 590

Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
595 600 605

Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
610 615 620

Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
625 630 635 640

Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
645 650

<210> SEQ ID NO 37
 <211> LENGTH: 1959
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant delta endotoxin
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1956)

<400> SEQUENCE: 37

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro 1 5 10 15	48
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn 20 25 30	96
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met 35 40 45	144
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp 50 55 60	192
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val 65 70 75 80	240
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu 85 90 95	288
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala 100 105 110	336
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser 115 120 125	384
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr 130 135 140	432
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser 145 150 155 160	480
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser 165 170 175	528
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val 180 185 190	576

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ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu 195 200 205	624
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu 210 215 220	672
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr 225 230 235 240	720
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg 245 250 255	768
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu 260 265 270	816
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp 275 280 285	864
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile 290 295 300	912
ttt acg gat cca att ttt tct ctt agg aca cca ctt gcg tac gga cca Phe Thr Asp Pro Ile Phe Ser Leu Arg Thr Pro Leu Ala Tyr Gly Pro 305 310 315 320	960
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp 325 330 335	1008
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe 340 345 350	1056
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg 355 360 365	1104
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp 370 375 380	1152
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 385 390 395 400	1200
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys 405 410 415	1248
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln 420 425 430	1296
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly 435 440 445	1344
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr 450 455 460	1392
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu 465 470 475 480	1440
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp 485 490 495	1488
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile	1536

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500	505	510	
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser 515 520 525			1584
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys 530 535 540			1632
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala 545 550 555 560			1680
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr 565 570 575			1728
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile 580 585 590			1776
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr 595 600 605			1824
ttt gat ctg gca act act aat tct aat atg ggg ttc tcg ggt gat aag Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys 610 615 620			1872
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile 625 630 635 640			1920
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu 645 650			1959

<210> SEQ ID NO 38

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 38

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Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
1           5           10           15

Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
20           25           30

Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
35           40           45

Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
50           55           60

Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
65           70           75           80

Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
85           90           95

Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
100          105          110

Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
115          120          125

Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
130          135          140

Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
145          150          155          160

Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser

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165								170					175				
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val		
			180					185					190				
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu		
		195					200					205					
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu		
	210					215					220						
Asp	Val	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr		
225					230					235					240		
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg		
			245						250					255			
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu		
			260					265					270				
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp		
		275					280					285					
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile		
	290					295						300					
Phe	Thr	Asp	Pro	Ile	Phe	Ser	Leu	Arg	Thr	Pro	Leu	Ala	Tyr	Gly	Pro		
305					310					315					320		
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp		
				325					330					335			
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe		
			340					345					350				
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg		
		355					360					365					
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp		
		370				375					380						
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val		
385					390					395					400		
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys		
				405					410					415			
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln		
			420					425					430				
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly		
		435					440					445					
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr		
		450				455					460						
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu		
465					470					475					480		
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp		
				485					490					495			
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile		
			500					505					510				
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser		
			515				520					525					
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys		
		530				535					540						
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala		
545					550					555					560		
Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr		
				565					570					575			
Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile		
			580					585					590				

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Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
595 600 605

Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
610 615 620

Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
625 630 635 640

Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
645 650

<210> SEQ ID NO 39
<211> LENGTH: 1959
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant delta endotoxin
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1956)

<400> SEQUENCE: 39

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro 1 5 10 15	48
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn 20 25 30	96
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met 35 40 45	144
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp 50 55 60	192
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val 65 70 75 80	240
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu 85 90 95	288
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala 100 105 110	336
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser 115 120 125	384
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr 130 135 140	432
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser 145 150 155 160	480
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser 165 170 175	528
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val 180 185 190	576
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu 195 200 205	624
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa	672

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Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu	
210						215					220					
gat	gtt	gct	gaa	ttt	tat	cat	aga	caa	tta	aaa	ctt	aca	caa	caa	tac	720
Asp	Val	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr	
225					230					235					240	
act	gac	cat	tgt	gtt	aat	tggt	tat	aat	gtt	gga	tta	aat	ggt	tta	aga	768
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg	
				245					250					255		
ggt	tca	act	tat	gat	gca	tggt	gtc	aaa	ttt	aac	cgt	ttt	cgc	aga	gaa	816
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu	
			260				265						270			
atg	act	tta	act	gta	tta	gat	cta	att	gta	ctt	ttc	cca	ttt	ttc	aat	864
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Phe	Asn	
		275				280						285				
att	ttg	ctt	tac	agt	aaa	ggg	gtt	aaa	aca	gaa	cta	aca	aga	gac	att	912
Ile	Leu	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile	
	290					295					300					
ttt	acg	gat	cca	att	ttt	tca	ctt	aat	act	ctt	cag	gag	tat	gga	cca	960
Phe	Thr	Asp	Pro	Ile	Phe	Ser	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro	
305					310					315					320	
act	ttt	ttg	agt	ata	gaa	aac	tct	att	cga	aaa	cct	cat	tta	ttt	gat	1008
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	
				325					330					335		
tat	tta	cag	ggg	att	gaa	ttt	cat	acg	cgt	ctt	caa	cct	ggt	tac	ttt	1056
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe	
		340						345					350			
ggg	aaa	gat	tct	ttc	aat	tat	tggt	tct	ggt	aat	tat	gta	gaa	act	aga	1104
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg	
	355						360					365				
cct	agt	ata	gga	tct	agt	aag	aca	att	act	tcc	cca	ttt	tat	gga	gat	1152
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp	
	370					375					380					
aaa	tct	act	gaa	cct	gta	caa	aag	cta	agc	ttt	gat	gga	caa	aaa	gtt	1200
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val	
	385				390					395					400	
tat	cga	act	ata	gct	aat	aca	gac	gta	gcg	gct	tggt	ccg	aat	ggt	aag	1248
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys	
			405					410						415		
gta	tat	tta	ggt	gtt	acg	aaa	gtt	gat	ttt	agt	caa	tat	gat	gat	caa	1296
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln	
		420					425						430			
aaa	aat	gaa	act	agt	aca	caa	aca	tat	gat	tca	aaa	aga	aac	aat	ggc	1344
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly	
	435					440						445				
cat	gta	agt	gca	cag	gat	tct	att	gac	caa	tta	ccg	cca	gaa	aca	aca	1392
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	
	450				455					460						
gat	gaa	cca	ctt	gaa	aaa	gca	tat	agt	cat	cag	ctt	aat	tac	gcg	gaa	1440
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu	
	465				470					475					480	
tgt	ttc	tta	atg	cag	gac	cgt	cgt	gga	aca	att	cca	ttt	ttt	act	tggt	1488
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp	
			485					490						495		
aca	cat	aga	agt	gta	gac	ttt	ttt	aat	aca	att	gat	gct	gaa	aag	att	1536
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile	
		500						505					510			
act	caa	ctt	cca	gta	gtg	aaa	gca	tat	gcc	ttg	tct	tca	ggt	gct	tcc	1584
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser	
	515						520					525				

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att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa	1632
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys	
530 535 540	
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca	1680
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala	
545 550 555 560	
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act	1728
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr	
565 570 575	
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc	1776
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile	
580 585 590	
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca	1824
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr	
595 600 605	
ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag	1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys	
610 615 620	
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc	1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile	
625 630 635 640	
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa	1959
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu	
645 650	

<210> SEQ ID NO 40

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 40

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser	
145 150 155 160	
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	
165 170 175	
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	
180 185 190	
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	

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195	200	205
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu 210 215 220		
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr 225 230 235 240		
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg 245 250 255		
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu 260 265 270		
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Phe Asn 275 280 285		
Ile Leu Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile 290 295 300		
Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro 305 310 315 320		
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp 325 330 335		
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe 340 345 350		
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg 355 360 365		
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp 370 375 380		
Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 385 390 395 400		
Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys 405 410 415		
Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln 420 425 430		
Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly 435 440 445		
His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr 450 455 460		
Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu 465 470 475 480		
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp 485 490 495		
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile 500 505 510		
Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser 515 520 525		
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys 530 535 540		
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala 545 550 555 560		
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr 565 570 575		
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile 580 585 590		
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr 595 600 605		
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys 610 615 620		

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Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
625 630 635 640

Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
645 650

<210> SEQ ID NO 41
<211> LENGTH: 1959
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant delta endotoxin
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1956)

<400> SEQUENCE: 41

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct	48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca	336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt	384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat	432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt	480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser	
145 150 155 160	
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt	528
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	
165 170 175	
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg	576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	
180 185 190	
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta	624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	
195 200 205	
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa	672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	
210 215 220	
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac	720
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr	
225 230 235 240	

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act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga	768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	
245 250 255	
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa	816
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	
260 265 270	
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat	864
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	
275 280 285	
att gtg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att	912
Ile Val Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	
290 295 300	
ttt acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca	960
Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro	
305 310 315 320	
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat	1008
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	
325 330 335	
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt	1056
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe	
340 345 350	
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga	1104
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg	
355 360 365	
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat	1152
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp	
370 375 380	
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt	1200
Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val	
385 390 395 400	
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag	1248
Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys	
405 410 415	
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa	1296
Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln	
420 425 430	
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc	1344
Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly	
435 440 445	
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca	1392
His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr	
450 455 460	
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa	1440
Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu	
465 470 475 480	
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg	1488
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp	
485 490 495	
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att	1536
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile	
500 505 510	
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc	1584
Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser	
515 520 525	
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa	1632
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys	
530 535 540	
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca	1680
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala	

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545	550	555	560	
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act				1728
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr				
	565	570	575	
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc				1776
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile				
	580	585	590	
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca				1824
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr				
	595	600	605	
ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag				1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys				
	610	615	620	
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc				1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile				
	625	630	635	640
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa				1959
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu				
	645	650		
 <210> SEQ ID NO 42				
<211> LENGTH: 652				
<212> TYPE: PRT				
<213> ORGANISM: Artificial sequence				
<220> FEATURE:				
<223> OTHER INFORMATION: Recombinant delta endotoxin				
 <400> SEQUENCE: 42				
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro				
1	5	10	15	
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn				
	20	25	30	
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met				
	35	40	45	
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp				
	50	55	60	
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val				
	65	70	75	80
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu				
	85	90	95	
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala				
	100	105	110	
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser				
	115	120	125	
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr				
	130	135	140	
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser				
	145	150	155	160
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser				
	165	170	175	
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val				
	180	185	190	
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu				
	195	200	205	
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu				
	210	215	220	
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr				

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225	230	235	240
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	245	250	255
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	260	265	270
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	275	280	285
Ile Val Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	290	295	300
Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro	305	310	315
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	325	330	335
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe	340	345	350
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg	355	360	365
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp	370	375	380
Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val	385	390	395
Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys	405	410	415
Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln	420	425	430
Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly	435	440	445
His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr	450	455	460
Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu	465	470	475
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp	485	490	495
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile	500	505	510
Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser	515	520	525
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys	530	535	540
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala	545	550	555
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr	565	570	575
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile	580	585	590
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr	595	600	605
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys	610	615	620
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile	625	630	635
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu	645	650	

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<210> SEQ ID NO 43
<211> LENGTH: 1959
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant delta endotoxin
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1956)

<400> SEQUENCE: 43

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct      48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
1          5          10          15

aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat      96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
20         25         30

cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg     144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
35         40         45

act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat     192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
50         55         60

gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta     240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
65         70         75

gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt     288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
85         90         95

aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca     336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
100        105        110

caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt     384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
115        120        125

aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat     432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
130        135        140

gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt     480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
145        150        155

aaa aga agc caa ggt cga ata agg gaa ctt ttt tct caa gca gaa agt     528
Lys Arg Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser
165        170        175

cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg     576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val
180        185        190

ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta     624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
195        200        205

tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa     672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
210        215        220

gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac     720
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr
225        230        235        240

act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga     768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg
245        250        255

ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa     816

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Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu	
			260					265					270			
atg	act	tta	act	gta	tta	gat	cta	att	gta	ctt	ttc	cca	ttt	tat	gat	864
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp	
		275					280					285				
att	cgg	tta	tac	tca	aaa	ggg	ggt	aaa	aca	gaa	cta	aca	aga	gac	att	912
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile	
	290					295					300					
ttt	acg	gat	cca	att	ttt	tca	ctt	aat	act	ctt	cag	gag	tat	gga	cca	960
Phe	Thr	Asp	Pro	Ile	Phe	Ser	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro	
305					310					315					320	
act	ttt	ttg	agt	ata	gaa	aac	tct	att	cga	aaa	cct	cat	tta	ttt	gat	1008
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	
				325					330						335	
tat	tta	cag	ggg	att	gaa	ttt	cat	acg	cgt	ctt	caa	cct	ggt	tac	ttt	1056
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe	
			340					345					350			
ggg	aaa	gat	tct	ttc	aat	tat	tgg	tct	ggt	aat	tat	gta	gaa	act	aga	1104
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg	
		355					360					365				
cct	agt	ata	gga	tct	agt	aag	aca	att	act	tcc	cca	ttt	tat	gga	gat	1152
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp	
		370				375					380					
aaa	tct	act	gaa	cct	gta	caa	aag	cta	agc	ttt	gat	gga	caa	aaa	gtt	1200
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val	
385					390					395					400	
tat	cga	act	ata	gct	aat	aca	gac	gta	gcg	gct	tgg	ccg	aat	ggt	aag	1248
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys	
				405					410						415	
gta	tat	tta	ggt	ggt	acg	aaa	gtt	gat	ttt	agt	caa	tat	gat	gat	caa	1296
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln	
		420						425					430			
aaa	aat	gaa	act	agt	aca	caa	aca	tat	gat	tca	aaa	aga	aac	aat	ggc	1344
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly	
		435					440					445				
cat	gta	agt	gca	cag	gat	tct	att	gac	caa	tta	ccg	cca	gaa	aca	aca	1392
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	
		450				455					460					
gat	gaa	cca	ctt	gaa	aaa	gca	tat	agt	cat	cag	ctt	aat	tac	gcg	gaa	1440
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu	
465					470					475					480	
tgt	ttc	tta	atg	cag	gac	cgt	cgt	gga	aca	att	cca	ttt	ttt	act	tgg	1488
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp	
				485					490					495		
aca	cat	aga	agt	gta	gac	ttt	ttt	aat	aca	att	gat	gct	gaa	aag	att	1536
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile	
		500						505					510			
act	caa	ctt	cca	gta	gtg	aaa	gca	tat	gcc	ttg	tct	tca	ggt	gct	tcc	1584
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser	
		515					520					525				
att	att	gaa	ggt	cca	gga	ttc	aca	gga	gga	aat	tta	cta	ttc	cta	aaa	1632
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys	
		530				535					540					
gaa	tct	agt	aat	tca	att	gct	aaa	ttt	aaa	gtt	aca	tta	aat	tca	gca	1680
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala	
545					550					555					560	
gcc	ttg	tta	caa	cga	tat	cgt	gta	aga	ata	cgc	tat	gct	tct	acc	act	1728
Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr	
				565					570					575		

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aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc 1776
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
      580              585              590

tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca 1824
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr
      595              600              605

ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag 1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
      610              615              620

aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc 1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
      625              630              635              640

tat ata gat aag ata gaa ttt atc cca gta caa ttg taa 1959
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
      645              650

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<210> SEQ ID NO 44
<211> LENGTH: 652
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant delta endotoxin

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<400> SEQUENCE: 44

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Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
1              5              10              15

Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
      20              25              30

Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
      35              40              45

Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
      50              55              60

Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
      65              70              75              80

Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
      85              90              95

Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
      100             105             110

Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
      115             120             125

Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
      130             135             140

Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
      145             150             155             160

Lys Arg Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser
      165             170             175

His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val
      180             185             190

Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
      195             200             205

Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
      210             215             220

Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr
      225             230             235             240

Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg
      245             250             255

Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu

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260					265					270					
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp
		275					280					285			
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile
	290					295					300				
Phe	Thr	Asp	Pro	Ile	Phe	Ser	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro
305					310					315					320
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp
			325						330					335	
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe
			340					345					350		
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg
	355						360					365			
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp
	370					375					380				
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val
385					390					395					400
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys
				405					410					415	
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln
			420					425					430		
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly
		435					440					445			
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr
	450					455					460				
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu
465					470					475					480
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp
			485						490					495	
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile
			500					505					510		
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser
		515					520					525			
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys
	530					535					540				
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala
545					550					555					560
Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr
			565					570					575		
Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile
		580					585					590			
Tyr	Ile	Asn	Lys	Thr	Met	Asn	Lys	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr
	595					600					605				
Phe	Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys
	610					615					620				
Asn	Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile
625					630					635					640
Tyr	Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu				
			645					650							

<210> SEQ ID NO 45

<211> LENGTH: 1959

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Recombinant delta endotoxin
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1956)

<400> SEQUENCE: 45

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct      48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
1          5          10          15

aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat      96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
20         25         30

cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg     144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
35         40         45

act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat     192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
50         55         60

gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta     240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
65         70         75

gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt     288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
85         90         95

aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca     336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
100        105        110

caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt     384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
115        120        125

aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat     432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
130        135        140

gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga aat     480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Asn
145        150        155

cca cac agc caa ggt cga ata agg gaa ctt ttt tct caa gca gaa agt     528
Pro His Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser
165        170        175

cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg     576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val
180        185        190

ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta     624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
195        200        205

tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa     672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
210        215        220

gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac     720
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr
225        230        235

act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga     768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg
245        250        255

ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa     816
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu
260        265        270

atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat     864
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp
275        280        285

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att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile 290 295 300	912
ttt acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro 305 310 315 320	960
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp 325 330 335	1008
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe 340 345 350	1056
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg 355 360 365	1104
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp 370 375 380	1152
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 385 390 395 400	1200
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys 405 410 415	1248
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln 420 425 430	1296
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly 435 440 445	1344
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr 450 455 460	1392
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu 465 470 475 480	1440
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp 485 490 495	1488
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile 500 505 510	1536
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser 515 520 525	1584
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys 530 535 540	1632
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala 545 550 555 560	1680
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr 565 570 575	1728
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile 580 585 590	1776
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr	1824

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595	600	605	
ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag			1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys			
610	615	620	
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc			1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile			
625	630	635	640
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa			1959
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu			
645	650		

<210> SEQ ID NO 46

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 46

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro			
1	5	10	15
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn			
20	25	30	
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met			
35	40	45	
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp			
50	55	60	
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val			
65	70	75	80
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu			
85	90	95	
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala			
100	105	110	
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser			
115	120	125	
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr			
130	135	140	
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Asn			
145	150	155	160
Pro His Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser			
165	170	175	
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val			
180	185	190	
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu			
195	200	205	
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu			
210	215	220	
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr			
225	230	235	240
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg			
245	250	255	
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu			
260	265	270	
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp			
275	280	285	
Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile			

-continued

290	295	300
Phe Thr Asp Pro Ile	Phe Ser Leu Asn Thr	Leu Gln Glu Tyr Gly Pro
305	310	315 320
Thr Phe Leu Ser Ile	Glu Asn Ser Ile Arg	Lys Pro His Leu Phe Asp
	325	330 335
Tyr Leu Gln Gly Ile	Glu Phe His Thr Arg	Leu Gln Pro Gly Tyr Phe
	340	345 350
Gly Lys Asp Ser Phe	Asn Tyr Trp Ser Gly	Asn Tyr Val Glu Thr Arg
	355	360 365
Pro Ser Ile Gly Ser	Ser Lys Thr Ile Thr	Ser Pro Phe Tyr Gly Asp
	370	375 380
Lys Ser Thr Glu Pro	Val Gln Lys Leu Ser	Phe Asp Gly Gln Lys Val
	385	390 395 400
Tyr Arg Thr Ile Ala	Asn Thr Asp Val Ala	Ala Trp Pro Asn Gly Lys
	405	410 415
Val Tyr Leu Gly Val	Thr Lys Val Asp Phe	Ser Gln Tyr Asp Asp Gln
	420	425 430
Lys Asn Glu Thr Ser	Thr Gln Thr Tyr Asp	Ser Lys Arg Asn Asn Gly
	435	440 445
His Val Ser Ala Gln	Asp Ser Ile Asp Gln	Leu Pro Pro Glu Thr Thr
	450	455 460
Asp Glu Pro Leu Glu	Lys Ala Tyr Ser His	Gln Leu Asn Tyr Ala Glu
	465	470 475 480
Cys Phe Leu Met Gln	Asp Arg Arg Gly Thr	Ile Pro Phe Phe Thr Trp
	485	490 495
Thr His Arg Ser Val	Asp Phe Phe Asn Thr	Ile Asp Ala Glu Lys Ile
	500	505 510
Thr Gln Leu Pro Val	Val Lys Ala Tyr Ala	Leu Ser Ser Gly Ala Ser
	515	520 525
Ile Ile Glu Gly Pro	Gly Phe Thr Gly Gly	Asn Leu Leu Phe Leu Lys
	530	535 540
Glu Ser Ser Asn Ser	Ile Ala Lys Phe Lys	Val Thr Leu Asn Ser Ala
	545	550 555 560
Ala Leu Leu Gln Arg	Tyr Arg Val Arg Ile	Arg Tyr Ala Ser Thr Thr
	565	570 575
Asn Leu Arg Leu Phe	Val Gln Asn Ser Asn	Asn Asp Phe Leu Val Ile
	580	585 590
Tyr Ile Asn Lys Thr	Met Asn Lys Asp Asp	Asp Leu Thr Tyr Gln Thr
	595	600 605
Phe Asp Leu Ala Thr	Thr Asn Ser Asn Met	Gly Phe Ser Gly Asp Lys
	610	615 620
Asn Glu Leu Ile Ile	Gly Ala Glu Ser Phe	Val Ser Asn Glu Lys Ile
	625	630 635 640
Tyr Ile Asp Lys Ile	Glu Phe Ile Pro Val	Gln Leu
	645	650

<210> SEQ ID NO 47

<211> LENGTH: 1959

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(1956)

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<400> SEQUENCE: 47

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct	48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca	336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt	384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat	432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt	480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser	
145 150 155 160	
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt	528
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	
165 170 175	
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg	576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	
180 185 190	
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta	624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	
195 200 205	
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa	672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	
210 215 220	
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac	720
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr	
225 230 235 240	
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga	768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	
245 250 255	
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa	816
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	
260 265 270	
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat	864
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	
275 280 285	
gtt cgg tta tac cca aaa ggg gtt aaa aca gaa cta aca aga gac att	912
Val Arg Leu Tyr Pro Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	
290 295 300	
ttt acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca	960

-continued

Phe 305	Thr	Asp	Pro	Ile	Phe 310	Ser	Leu	Asn	Thr	Leu 315	Gln	Glu	Tyr	Gly	Pro 320	
act	ttt	ttg	agt	ata	gaa	aac	tct	att	cga	aaa	cct	cat	tta	ttt	gat	1008
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	
				325					330					335		
tat	tta	cag	ggg	att	gaa	ttt	cat	acg	cgt	ctt	caa	cct	ggt	tac	ttt	1056
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe	
			340					345					350			
ggg	aaa	gat	tct	ttc	aat	tat	tgg	tct	ggt	aat	tat	gta	gaa	act	aga	1104
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg	
		355					360					365				
cct	agt	ata	gga	tct	agt	aag	aca	att	act	tcc	cca	ttt	tat	gga	gat	1152
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp	
		370				375					380					
aaa	tct	act	gaa	cct	gta	caa	aag	cta	agc	ttt	gat	gga	caa	aaa	gtt	1200
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val	
		385			390					395				400		
tat	cga	act	ata	gct	aat	aca	gac	gta	gcg	gct	tgg	ccg	aat	ggt	aag	1248
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys	
			405					410						415		
gta	tat	tta	ggt	gtt	acg	aaa	gtt	gat	ttt	agt	caa	tat	gat	gat	caa	1296
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln	
		420						425					430			
aaa	aat	gaa	act	agt	aca	caa	aca	tat	gat	tca	aaa	aga	aac	aat	ggc	1344
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly	
		435				440						445				
cat	gta	agt	gca	cag	gat	tct	att	gac	caa	tta	ccg	cca	gaa	aca	aca	1392
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	
		450				455				460						
gat	gaa	cca	ctt	gaa	aaa	gca	tat	agt	cat	cag	ctt	aat	tac	gcg	gaa	1440
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu	
		465			470					475				480		
tgt	ttc	tta	atg	cag	gac	cgt	cgt	gga	aca	att	cca	ttt	ttt	act	tgg	1488
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp	
			485					490						495		
aca	cat	aga	agt	gta	gac	ttt	ttt	aat	aca	att	gat	gct	gaa	aag	att	1536
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile	
		500						505					510			
act	caa	ctt	cca	gta	gtg	aaa	gca	tat	gcc	ttg	tct	tca	ggt	gct	tcc	1584
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser	
		515				520						525				
att	att	gaa	ggt	cca	gga	ttc	aca	gga	gga	aat	tta	cta	ttc	cta	aaa	1632
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys	
		530				535					540					
gaa	tct	agt	aat	tca	att	gct	aaa	ttt	aaa	gtt	aca	tta	aat	tca	gca	1680
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala	
		545			550				555					560		
gcc	ttg	tta	caa	cga	tat	cgt	gta	aga	ata	cgc	tat	gct	tct	acc	act	1728
Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr	
			565					570						575		
aac	tta	cga	ctt	ttt	gtg	caa	aat	tca	aac	aat	gat	ttt	ctt	gtc	atc	1776
Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile	
			580					585					590			
tac	att	aat	aaa	act	atg	aat	aaa	gat	gat	gat	tta	aca	tat	caa	aca	1824
Tyr	Ile	Asn	Lys	Thr	Met	Asn	Lys	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr	
		595				600						605				
ttt	gat	ctc	gca	act	act	aat	tct	aat	atg	ggg	ttc	tcg	ggt	gat	aag	1872
Phe	Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys	
		610				615					620					

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aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc      1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
625                      630                      635                      640

```

```

tat ata gat aag ata gaa ttt atc cca gta caa ttg taa      1959
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
                      645                      650

```

<210> SEQ ID NO 48

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 48

```

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
1                      5                      10                      15

```

```

Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
20                      25                      30

```

```

Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
35                      40                      45

```

```

Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
50                      55                      60

```

```

Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
65                      70                      75                      80

```

```

Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
85                      90                      95

```

```

Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
100                      105                      110

```

```

Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
115                      120                      125

```

```

Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
130                      135                      140

```

```

Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
145                      150                      155                      160

```

```

Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser
165                      170                      175

```

```

His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val
180                      185                      190

```

```

Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
195                      200                      205

```

```

Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
210                      215                      220

```

```

Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr
225                      230                      235                      240

```

```

Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg
245                      250                      255

```

```

Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu
260                      265                      270

```

```

Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp
275                      280                      285

```

```

Val Arg Leu Tyr Pro Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile
290                      295                      300

```

```

Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro
305                      310                      315                      320

```

```

Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp

```

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325										330										335									
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe														
			340						345						350														
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg														
		355					360					365																	
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp														
	370					375					380																		
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val														
	385				390					395					400														
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys														
				405					410					415															
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln														
		420						425					430																
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly														
		435					440					445																	
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr														
		450				455					460																		
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu														
	465				470					475					480														
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp														
			485					490						495															
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile														
			500					505					510																
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser														
		515					520					525																	
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys														
	530					535					540																		
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala														
	545				550					555					560														
Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr														
			565					570						575															
Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile														
		580					585					590																	
Tyr	Ile	Asn	Lys	Thr	Met	Asn	Lys	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr														
		595					600					605																	
Phe	Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys														
	610					615					620																		
Asn	Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile														
	625				630					635				640															
Tyr	Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu																		
			645					650																					

<210> SEQ ID NO 49

<211> LENGTH: 1959

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(1956)

<400> SEQUENCE: 49

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
1 5 10 15

48

-continued

aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca	336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt	384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat	432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga aat	480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Asn	
145 150 155 160	
cca cac agc caa ggt cga ata agg gaa ctt ttt tct caa gca gaa agt	528
Pro His Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	
165 170 175	
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg	576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	
180 185 190	
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta	624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	
195 200 205	
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa	672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	
210 215 220	
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac	720
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr	
225 230 235 240	
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga	768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	
245 250 255	
ggg tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa	816
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	
260 265 270	
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat	864
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	
275 280 285	
gtt cgg tta tac cca aaa ggg gtt aaa aca gaa cta aca aga gac att	912
Val Arg Leu Tyr Pro Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	
290 295 300	
ttt acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca	960
Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro	
305 310 315 320	
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat	1008
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	
325 330 335	

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tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe 340 345 350	1056
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg 355 360 365	1104
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp 370 375 380	1152
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 385 390 395 400	1200
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys 405 410 415	1248
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln 420 425 430	1296
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly 435 440 445	1344
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr 450 455 460	1392
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu 465 470 475 480	1440
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp 485 490 495	1488
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile 500 505 510	1536
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser 515 520 525	1584
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys 530 535 540	1632
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala 545 550 555 560	1680
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr 565 570 575	1728
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile 580 585 590	1776
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr 595 600 605	1824
ttt gat ctg gca act act aat tct aat atg ggg ttc tcg ggt gat aag Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys 610 615 620	1872
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile 625 630 635 640	1920
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu	1959

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645	650
<210> SEQ ID NO 50	
<211> LENGTH: 652	
<212> TYPE: PRT	
<213> ORGANISM: Artificial sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: Recombinant delta endotoxin	
<400> SEQUENCE: 50	
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Asn	
145 150 155 160	
Pro His Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	
165 170 175	
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	
180 185 190	
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	
195 200 205	
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	
210 215 220	
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr	
225 230 235 240	
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	
245 250 255	
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	
260 265 270	
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	
275 280 285	
Val Arg Leu Tyr Pro Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	
290 295 300	
Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro	
305 310 315 320	
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	
325 330 335	
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe	
340 345 350	
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg	

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355					360					365					
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp
370						375					380				
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val
385						390					395				400
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys
				405					410					415	
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln
			420					425					430		
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly
			435				440					445			
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr
			450				455					460			
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu
465						470					475				480
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp
				485					490					495	
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile
			500					505					510		
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser
			515				520					525			
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys
			530				535					540			
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala
545						550					555				560
Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr
				565					570					575	
Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile
			580					585					590		
Tyr	Ile	Asn	Lys	Thr	Met	Asn	Lys	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr
			595				600					605			
Phe	Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys
			610				615					620			
Asn	Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile
625						630					635				640
Tyr	Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu				
				645					650						

<210> SEQ ID NO 51

<211> LENGTH: 1956

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(1953)

<400> SEQUENCE: 51

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct	48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	

-continued

35	40	45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp 50 55 60			192
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val 65 70 75 80			240
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu 85 90 95			288
aac act ata tgg cca agt gaa gac cca tgg aag gct ttt atg gca caa Asn Thr Ile Trp Pro Ser Glu Asp Pro Trp Lys Ala Phe Met Ala Gln 100 105 110			336
gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt aaa Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser Lys 115 120 125			384
gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat gtt Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr Val 130 135 140			432
aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt aaa Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser Lys 145 150 155 160			480
aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt cat Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His 165 170 175			528
ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg ctg Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val Leu 180 185 190			576
ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta tta Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu Leu 195 200 205			624
aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa gat Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu Asp 210 215 220			672
gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac act Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr Thr 225 230 235 240			720
gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga ggt Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg Gly 245 250 255			768
tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa atg Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Glu Met 260 265 270			816
act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat att Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp Ile 275 280 285			864
cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att ttt Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile Phe 290 295 300			912
acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca act Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro Thr 305 310 315 320			960
ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat tat Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp Tyr 325 330 335			1008
tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt ggg Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe Gly 340 345 350			1056
aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga cct			1104

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Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg	Pro		
	355						360					365					
agt	ata	gga	tct	agt	aag	aca	att	act	tcc	cca	ttt	tat	gga	gat	aaa	1152	
Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp	Lys		
	370					375					380						
tct	act	gaa	cct	gta	caa	aag	cta	agc	ttt	gat	gga	caa	aaa	gtt	tat	1200	
Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val	Tyr		
	385				390				395					400			
cga	act	ata	gct	aat	aca	gac	gta	gcg	gct	tggt	ccg	aat	ggg	aag	gta	1248	
Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys	Val		
			405					410					415				
tat	tta	ggg	gtt	acg	aaa	gtt	gat	ttt	agt	caa	tat	gat	gat	caa	aaa	1296	
Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln	Lys		
		420					425					430					
aat	gaa	act	agt	aca	caa	aca	tat	gat	tca	aaa	aga	aac	aat	ggc	cat	1344	
Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly	His		
	435					440					445						
gta	agt	gca	cag	gat	tct	att	gac	caa	tta	ccg	cca	gaa	aca	aca	gat	1392	
Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	Asp		
	450					455				460							
gaa	cca	ctt	gaa	aaa	gca	tat	agt	cat	cag	ctt	aat	tac	gcg	gaa	tgt	1440	
Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu	Cys		
	465			470					475					480			
ttc	tta	atg	cag	gac	cgt	cgt	gga	aca	att	cca	ttt	ttt	act	tggt	aca	1488	
Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp	Thr		
			485					490					495				
cat	aga	agt	gta	gac	ttt	ttt	aat	aca	att	gat	gct	gaa	aag	att	act	1536	
His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile	Thr		
	500						505					510					
caa	ctt	cca	gta	gtg	aaa	gca	tat	gcc	ttg	tct	tca	ggg	gct	tcc	att	1584	
Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser	Ile		
	515					520					525						
att	gaa	ggg	cca	gga	ttc	aca	gga	gga	aat	tta	cta	ttc	cta	aaa	gaa	1632	
Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys	Glu		
	530				535					540							
tct	agt	aat	tca	att	gct	aaa	ttt	aaa	gtt	aca	tta	aat	tca	gca	gcc	1680	
Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala	Ala		
	545			550					555					560			
ttg	tta	caa	cga	tat	cgt	gta	aga	ata	cggt	tat	gct	tct	acc	act	aac	1728	
Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr	Asn		
			565					570					575				
tta	cga	ctt	ttt	gtg	caa	aat	tca	aac	aat	gat	ttt	ctt	gtc	atc	tac	1776	
Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile	Tyr		
		580					585					590					
att	aat	aaa	act	atg	aat	aaa	gat	gat	gat	tta	aca	tat	caa	aca	ttt	1824	
Ile	Asn	Lys	Thr	Met	Asn	Lys	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr	Phe		
	595					600					605						
gat	ctc	gca	act	act	aat	tct	aat	atg	ggg	ttc	tcg	ggg	gat	aag	aat	1872	
Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys	Asn		
	610					615				620							
gaa	ctt	ata	ata	gga	gca	gaa	tct	ttc	gtt	tct	aat	gaa	aaa	atc	tat	1920	
Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile	Tyr		
	625			630				635						640			
ata	gat	aag	ata	gaa	ttt	atc	cca	gta	caa	ttg	taa					1956	
Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu							
			645					650									

<210> SEQ ID NO 52

<211> LENGTH: 651

<212> TYPE: PRT

-continued

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 52

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Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
 1           5           10           15

Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
 20           25           30

Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
 35           40           45

Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
 50           55           60

Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
 65           70           75           80

Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
 85           90           95

Asn Thr Ile Trp Pro Ser Glu Asp Pro Trp Lys Ala Phe Met Ala Gln
100          105          110

Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser Lys
115          120          125

Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr Val
130          135          140

Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser Lys
145          150          155          160

Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His
165          170          175

Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val Leu
180          185          190

Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu Leu
195          200          205

Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu Asp
210          215          220

Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr Thr
225          230          235          240

Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg Gly
245          250          255

Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu Met
260          265          270

Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp Ile
275          280          285

Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile Phe
290          295          300

Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro Thr
305          310          315          320

Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp Tyr
325          330          335

Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe Gly
340          345          350

Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg Pro
355          360          365

Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp Lys
370          375          380

Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val Tyr

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385	390	395	400
Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys Val	405	410	415
Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln Lys	420	425	430
Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly His	435	440	445
Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp	450	455	460
Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu Cys	465	470	475
Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp Thr	485	490	495
His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile Thr	500	505	510
Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser Ile	515	520	525
Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys Glu	530	535	540
Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala Ala	545	550	555
Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr Asn	565	570	575
Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile Tyr	580	585	590
Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr Phe	595	600	605
Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys Asn	610	615	620
Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile Tyr	625	630	635
Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu	645	650	

<210> SEQ ID NO 53

<211> LENGTH: 1959

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(1956)

<400> SEQUENCE: 53

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct	48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	

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gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val 65 70 75 80	240
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu 85 90 95	288
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala 100 105 110	336
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser 115 120 125	384
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr 130 135 140	432
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser 145 150 155 160	480
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser 165 170 175	528
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc gga ttc gaa gtg His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Gly Phe Glu Val 180 185 190	576
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu 195 200 205	624
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu 210 215 220	672
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr 225 230 235 240	720
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg 245 250 255	768
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu 260 265 270	816
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp 275 280 285	864
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile 290 295 300	912
ttt acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro 305 310 315 320	960
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp 325 330 335	1008
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe 340 345 350	1056
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg 355 360 365	1104
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp 370 375 380	1152

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aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 385 390 395 400	1200
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys 405 410 415	1248
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln 420 425 430	1296
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly 435 440 445	1344
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr 450 455 460	1392
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu 465 470 475 480	1440
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp 485 490 495	1488
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile 500 505 510	1536
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser 515 520 525	1584
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys 530 535 540	1632
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala 545 550 555 560	1680
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr 565 570 575	1728
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile 580 585 590	1776
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr 595 600 605	1824
ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys 610 615 620	1872
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile 625 630 635 640	1920
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu 645 650	1959

<210> SEQ ID NO 54

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 54

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Met	Asn	Pro	Asn	Asn	Arg	Ser	Glu	His	Asp	Thr	Ile	Lys	Val	Thr	Pro
1			5						10					15	
Asn	Ser	Glu	Leu	Gln	Thr	Asn	His	Asn	Gln	Tyr	Pro	Leu	Ala	Asp	Asn
		20						25					30		
Pro	Asn	Ser	Thr	Leu	Glu	Glu	Leu	Asn	Tyr	Lys	Glu	Phe	Leu	Arg	Met
		35					40					45			
Thr	Glu	Asp	Ser	Ser	Thr	Glu	Val	Leu	Asp	Asn	Ser	Thr	Val	Lys	Asp
	50					55					60				
Ala	Val	Gly	Thr	Gly	Ile	Ser	Val	Val	Gly	Gln	Ile	Leu	Gly	Val	Val
65					70					75					80
Gly	Val	Pro	Phe	Ala	Gly	Ala	Leu	Thr	Ser	Phe	Tyr	Gln	Ser	Phe	Leu
				85					90					95	
Asn	Thr	Ile	Trp	Pro	Ser	Asp	Ala	Asp	Pro	Trp	Lys	Ala	Phe	Met	Ala
			100					105					110		
Gln	Val	Glu	Val	Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser
		115					120					125			
Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr
	130					135					140				
Val	Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Thr	Pro	Leu	Ser	Leu	Arg	Ser
145					150					155					160
Lys	Arg	Ser	Gln	Asp	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser
				165					170					175	
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Gly	Phe	Glu	Val
			180					185					190		
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu
		195					200					205			
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu
	210					215					220				
Asp	Val	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr
225					230					235					240
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg
			245					250						255	
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu
			260					265					270		
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp
		275					280					285			
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile
	290					295					300				
Phe	Thr	Asp	Pro	Ile	Phe	Ser	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro
305					310					315					320
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp
			325						330					335	
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe
			340					345					350		
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg
		355					360					365			
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp
		370				375					380				
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val
385					390					395					400
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys
				405					410					415	
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln

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420					425					430					
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly
	435						440					445			
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr
	450					455					460				
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu
	465					470					475				480
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp
				485					490					495	
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile
			500					505					510		
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser
		515					520					525			
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys
	530					535					540				
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala
	545					550					555				560
Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr
			565						570					575	
Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile
			580					585					590		
Tyr	Ile	Asn	Lys	Thr	Met	Asn	Lys	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr
		595					600					605			
Phe	Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys
	610					615					620				
Asn	Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile
	625					630					635				640
Tyr	Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu				
			645					650							

<210> SEQ ID NO 55

<211> LENGTH: 1956

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(1953)

<400> SEQUENCE: 55

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct	48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	

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85	90	95	
aac act ata tgg cca agt gaa gac cca tgg aag gct ttt atg gca caa			336
Asn Thr Ile Trp Pro Ser Glu Asp Pro Trp Lys Ala Phe Met Ala Gln			
100	105	110	
gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt aaa			384
Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser Lys			
115	120	125	
gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat gtt			432
Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr Val			
130	135	140	
aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga aat cca			480
Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Asn Pro			
145	150	155	160
cac agc caa ggt cga ata agg gaa ctt ttt tct caa gca gaa agt cat			528
His Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His			
165	170	175	
ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg ctg			576
Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val Leu			
180	185	190	
ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta tta			624
Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu Leu			
195	200	205	
aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa gat			672
Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu Asp			
210	215	220	
gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac act			720
Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr Thr			
225	230	235	240
gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga ggt			768
Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg Gly			
245	250	255	
tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa atg			816
Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu Met			
260	265	270	
act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat att			864
Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp Ile			
275	280	285	
cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att ttt			912
Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile Phe			
290	295	300	
acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca act			960
Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro Thr			
305	310	315	320
ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat tat			1008
Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp Tyr			
325	330	335	
tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt ggg			1056
Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe Gly			
340	345	350	
aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga cct			1104
Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg Pro			
355	360	365	
agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat aaa			1152
Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp Lys			
370	375	380	
tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt tat			1200
Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val Tyr			
385	390	395	400
cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag gta			1248

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Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys	Val		
				405					410					415			
tat	tta	ggt	gtt	acg	aaa	gtt	gat	ttt	agt	caa	tat	gat	gat	caa	aaa	1296	
Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln	Lys		
			420					425					430				
aat	gaa	act	agt	aca	caa	aca	tat	gat	tca	aaa	aga	aac	aat	ggc	cat	1344	
Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly	His		
			435				440					445					
gta	agt	gca	cag	gat	tct	att	gac	caa	tta	ccg	cca	gaa	aca	aca	gat	1392	
Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	Asp		
			450			455					460						
gaa	cca	ctt	gaa	aaa	gca	tat	agt	cat	cag	ctt	aat	tac	gcg	gaa	tgt	1440	
Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu	Cys		
465				470					475					480			
ttc	tta	atg	cag	gac	cgt	cgt	gga	aca	att	cca	ttt	ttt	act	tggt	aca	1488	
Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp	Thr		
			485					490					495				
cat	aga	agt	gta	gac	ttt	ttt	aat	aca	att	gat	gct	gaa	aag	att	act	1536	
His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile	Thr		
			500				505					510					
caa	ctt	cca	gta	gtg	aaa	gca	tat	gcc	ttg	tct	tca	ggt	gct	tcc	att	1584	
Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser	Ile		
			515			520						525					
att	gaa	ggt	cca	gga	ttc	aca	gga	gga	aat	tta	cta	ttc	cta	aaa	gaa	1632	
Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys	Glu		
		530			535					540							
tct	agt	aat	tca	att	gct	aaa	ttt	aaa	gtt	aca	tta	aat	tca	gca	gcc	1680	
Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala	Ala		
545				550					555					560			
ttg	tta	caa	cga	tat	cgt	gta	aga	ata	cgc	tat	gct	tct	acc	act	aac	1728	
Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr	Asn		
			565					570					575				
tta	cga	ctt	ttt	gtg	caa	aat	tca	aac	aat	gat	ttt	ctt	gtc	atc	tac	1776	
Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile	Tyr		
			580				585					590					
att	aat	aaa	act	atg	aat	aaa	gat	gat	gat	tta	aca	tat	caa	aca	ttt	1824	
Ile	Asn	Lys	Thr	Met	Asn	Lys	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr	Phe		
		595				600						605					
gat	ctc	gca	act	act	aat	tct	aat	atg	ggg	ttc	tcg	ggt	gat	aag	aat	1872	
Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys	Asn		
		610			615				620								
gaa	ctt	ata	ata	gga	gca	gaa	tct	ttc	gtt	tct	aat	gaa	aaa	atc	tat	1920	
Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile	Tyr		
625				630				635						640			
ata	gat	aag	ata	gaa	ttt	atc	cca	gta	caa	ttg	taa					1956	
Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu							
			645					650									

<210> SEQ ID NO 56

<211> LENGTH: 651

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 56

Met	Asn	Pro	Asn	Asn	Arg	Ser	Glu	His	Asp	Thr	Ile	Lys	Val	Thr	Pro
1			5						10					15	

Asn	Ser	Glu	Leu	Gln	Thr	Asn	His	Asn	Gln	Tyr	Pro	Leu	Ala	Asp	Asn
		20						25					30		

-continued

Pro	Asn	Ser	Thr	Leu	Glu	Glu	Leu	Asn	Tyr	Lys	Glu	Phe	Leu	Arg	Met
	35						40					45			
Thr	Glu	Asp	Ser	Ser	Thr	Glu	Val	Leu	Asp	Asn	Ser	Thr	Val	Lys	Asp
	50					55				60					
Ala	Val	Gly	Thr	Gly	Ile	Ser	Val	Val	Gly	Gln	Ile	Leu	Gly	Val	Val
65					70					75				80	
Gly	Val	Pro	Phe	Ala	Gly	Ala	Leu	Thr	Ser	Phe	Tyr	Gln	Ser	Phe	Leu
			85						90					95	
Asn	Thr	Ile	Trp	Pro	Ser	Glu	Asp	Pro	Trp	Lys	Ala	Phe	Met	Ala	Gln
		100						105					110		
Val	Glu	Val	Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser	Lys
	115					120						125			
Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr	Val
	130					135					140				
Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Thr	Pro	Leu	Ser	Leu	Arg	Asn	Pro
145				150					155					160	
His	Ser	Gln	Gly	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser	His
			165						170					175	
Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val	Leu
		180					185						190		
Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	Leu
	195					200						205			
Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu	Asp
	210					215					220				
Val	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr	Thr
225					230					235				240	
Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg	Gly
			245						250					255	
Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu	Met
		260						265						270	
Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp	Ile
	275					280						285			
Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile	Phe
	290					295					300				
Thr	Asp	Pro	Ile	Phe	Ser	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro	Thr
305					310					315				320	
Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	Tyr
			325						330					335	
Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe	Gly
		340						345						350	
Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg	Pro
	355						360					365			
Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp	Lys
	370					375					380				
Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val	Tyr
385					390					395				400	
Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys	Val
			405						410					415	
Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln	Lys
		420						425					430		
Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly	His
	435						440					445			
Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	Asp

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450	455	460
Glu Pro Leu Glu Lys	Ala Tyr Ser His Gln	Leu Asn Tyr Ala Glu Cys
465	470	475 480
Phe Leu Met Gln Asp	Arg Arg Gly Thr Ile	Pro Phe Phe Thr Trp Thr
	485	490 495
His Arg Ser Val Asp	Phe Phe Asn Thr Ile	Asp Ala Glu Lys Ile Thr
	500	505 510
Gln Leu Pro Val Val	Lys Ala Tyr Ala Leu Ser	Ser Gly Ala Ser Ile
	515	520 525
Ile Glu Gly Pro Gly	Phe Thr Gly Gly Asn Leu	Leu Phe Leu Lys Glu
	530	535 540
Ser Ser Asn Ser Ile	Ala Lys Phe Lys Val Thr	Leu Asn Ser Ala Ala
545	550	555 560
Leu Leu Gln Arg Tyr	Arg Val Arg Ile Arg	Tyr Ala Ser Thr Thr Asn
	565	570 575
Leu Arg Leu Phe Val	Gln Asn Ser Asn Asn Asp	Phe Leu Val Ile Tyr
	580	585 590
Ile Asn Lys Thr Met	Asn Lys Asp Asp Asp	Leu Thr Tyr Gln Thr Phe
	595	600 605
Asp Leu Ala Thr Thr	Asn Ser Asn Met Gly Phe	Ser Gly Asp Lys Asn
	610	615 620
Glu Leu Ile Ile Gly	Ala Glu Ser Phe Val Ser	Asn Glu Lys Ile Tyr
625	630	635 640
Ile Asp Lys Ile Glu	Phe Ile Pro Val Gln Leu	
	645	650

<210> SEQ ID NO 57

<211> LENGTH: 1956

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(1953)

<400> SEQUENCE: 57

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct	48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
aac act ata tgg cca agt gaa gac cca tgg aag gct ttt atg gca caa	336
Asn Thr Ile Trp Pro Ser Glu Asp Pro Trp Lys Ala Phe Met Ala Gln	
100 105 110	

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ggt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt aaa	384
Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser Lys	
115 120 125	
gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat gtt	432
Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr Val	
130 135 140	
aat gcg tta aat tcc tgg aag aaa ttt cac cat tct cgt cgt tct aaa	480
Asn Ala Leu Asn Ser Trp Lys Lys Phe His His Ser Arg Arg Ser Lys	
145 150 155 160	
aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt cat	528
Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His	
165 170 175	
ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg ctg	576
Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val Leu	
180 185 190	
ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta tta	624
Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu Leu	
195 200 205	
aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa gat	672
Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu Asp	
210 215 220	
ggt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac act	720
Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr Thr	
225 230 235 240	
gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga ggt	768
Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg Gly	
245 250 255	
tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa atg	816
Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu Met	
260 265 270	
act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat att	864
Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp Ile	
275 280 285	
cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att ttt	912
Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile Phe	
290 295 300	
acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca act	960
Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro Thr	
305 310 315 320	
ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat tat	1008
Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp Tyr	
325 330 335	
tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt ggg	1056
Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe Gly	
340 345 350	
aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga cct	1104
Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg Pro	
355 360 365	
agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat aaa	1152
Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp Lys	
370 375 380	
tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt tat	1200
Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val Tyr	
385 390 395 400	
cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag gta	1248
Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys Val	
405 410 415	
tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa aaa	1296
Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln Lys	
420 425 430	

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aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc cat 1344
Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly His
      435                440                445

gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca gat 1392
Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp
      450                455                460

gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa tgt 1440
Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu Cys
      465                470                475                480

ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg aca 1488
Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp Thr
      485                490                495

cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att act 1536
His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile Thr
      500                505                510

caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc att 1584
Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser Ile
      515                520                525

att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa gaa 1632
Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys Glu
      530                535                540

tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca gcc 1680
Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala Ala
      545                550                555                560

ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act aac 1728
Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr Asn
      565                570                575

tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc tac 1776
Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile Tyr
      580                585                590

att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca ttt 1824
Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr Phe
      595                600                605

gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag aat 1872
Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys Asn
      610                615                620

gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc tat 1920
Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile Tyr
      625                630                635                640

ata gat aag ata gaa ttt atc cca gta caa ttg taa 1956
Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
      645                650

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<210> SEQ ID NO 58

<211> LENGTH: 651

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 58

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Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
1      5      10      15

Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
20     25     30

Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
35     40     45

Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
50     55     60

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Ala	Val	Gly	Thr	Gly	Ile	Ser	Val	Val	Gly	Gln	Ile	Leu	Gly	Val	Val	65	70	75	80
Gly	Val	Pro	Phe	Ala	Gly	Ala	Leu	Thr	Ser	Phe	Tyr	Gln	Ser	Phe	Leu	85	90	95	
Asn	Thr	Ile	Trp	Pro	Ser	Glu	Asp	Pro	Trp	Lys	Ala	Phe	Met	Ala	Gln	100	105	110	
Val	Glu	Val	Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser	Lys	115	120	125	
Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr	Val	130	135	140	
Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Phe	His	His	Ser	Arg	Arg	Ser	Lys	145	150	155	160
Arg	Ser	Gln	Asp	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser	His	165	170	175	
Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val	Leu	180	185	190	
Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	Leu	195	200	205	
Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu	Asp	210	215	220	
Val	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr	Thr	225	230	235	240
Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg	Gly	245	250	255	
Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu	Met	260	265	270	
Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp	Ile	275	280	285	
Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile	Phe	290	295	300	
Thr	Asp	Pro	Ile	Phe	Ser	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro	Thr	305	310	315	320
Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	Tyr	325	330	335	
Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe	Gly	340	345	350	
Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg	Pro	355	360	365	
Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp	Lys	370	375	380	
Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val	Tyr	385	390	395	400
Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys	Val	405	410	415	
Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln	Lys	420	425	430	
Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly	His	435	440	445	
Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	Asp	450	455	460	
Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu	Cys	465	470	475	480
Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp	Thr				

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485										490					495				
His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile	Thr				
			500					505					510						
Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser	Ile				
		515					520					525							
Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys	Glu				
	530					535					540								
Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala	Ala				
545					550					555					560				
Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr	Asn				
			565					570						575					
Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile	Tyr				
		580					585						590						
Ile	Asn	Lys	Thr	Met	Asn	Lys	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr	Phe				
	595						600					605							
Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys	Asn				
	610				615						620								
Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile	Tyr				
625					630					635					640				
Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu									
			645					650											

<210> SEQ ID NO 59

<211> LENGTH: 1959

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(1956)

<400> SEQUENCE: 59

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct	48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca	336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt	384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat	432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	

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130	135	140	
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt			480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser			
145	150	155	160
aaa aga agc caa ggt cga ata agg gaa ctt ttt tct caa gca gaa agt			528
Lys Arg Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser			
	165	170	175
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg			576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val			
	180	185	190
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta			624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu			
	195	200	205
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa			672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu			
	210	215	220
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac			720
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr			
	225	230	235
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga			768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg			
	245	250	255
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa			816
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu			
	260	265	270
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat			864
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp			
	275	280	285
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att			912
Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile			
	290	295	300
ttt acg gat cca att ttt acc ctt aat aca cta cag aag tac gga cca			960
Phe Thr Asp Pro Ile Phe Thr Leu Asn Thr Leu Gln Lys Tyr Gly Pro			
	305	310	315
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat			1008
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp			
	325	330	335
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt			1056
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe			
	340	345	350
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga			1104
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg			
	355	360	365
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat			1152
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp			
	370	375	380
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt			1200
Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val			
	385	390	395
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag			1248
Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys			
	405	410	415
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa			1296
Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln			
	420	425	430
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc			1344
Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly			
	435	440	445
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca			1392

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His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr	
450 455 460	
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa	1440
Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu	
465 470 475 480	
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg	1488
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp	
485 490 495	
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att	1536
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile	
500 505 510	
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc	1584
Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser	
515 520 525	
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa	1632
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys	
530 535 540	
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca	1680
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala	
545 550 555 560	
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act	1728
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr	
565 570 575	
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc	1776
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile	
580 585 590	
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca	1824
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr	
595 600 605	
ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag	1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys	
610 615 620	
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc	1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile	
625 630 635 640	
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa	1959
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu	
645 650	

<210> SEQ ID NO 60

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 60

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	

Asn	Thr	Ile	Trp	Pro	Ser	Asp	Ala	Asp	Pro	Trp	Lys	Ala	Phe	Met	Ala
			100					105					110		
Gln	Val	Glu	Val	Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser
		115					120					125			
Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr
		130				135					140				
Val	Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Thr	Pro	Leu	Ser	Leu	Arg	Ser
		145			150				170	155					160
Lys	Arg	Ser	Gln	Gly	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser
			165											175	
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val
			180					185					190		
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu
		195					200					205			
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu
		210				215					220				
Asp	Val	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr
					230					235					240
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg
			245					250						255	
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu
			260					265					270		
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp
		275					280					285			
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile
		290				295					300				
Phe	Thr	Asp	Pro	Ile	Phe	Thr	Leu	Asn	Thr	Leu	Gln	Lys	Tyr	Gly	Pro
					310					315					320
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp
			325						330					335	
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe
			340					345					350		
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg
		355					360					365			
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp
		370				375					380				
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val
					390					395					400
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys
				405					410					415	
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln
			420					425					430		
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly
		435					440					445			
His	Val	Ser	Ala	Gln</											

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515	520	525	
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys 530 535 540			
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala 545 550 555 560			
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr 565 570 575			
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile 580 585 590			
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr 595 600 605			
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys 610 615 620			
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile 625 630 635 640			
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu 645 650			
<210> SEQ ID NO 61			
<211> LENGTH: 1959			
<212> TYPE: DNA			
<213> ORGANISM: Artificial sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Recombinant delta endotoxin			
<220> FEATURE:			
<221> NAME/KEY: CDS			
<222> LOCATION: (1)..(1956)			
<400> SEQUENCE: 61			
atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro 1 5 10 15			48
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn 20 25 30			96
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met 35 40 45			144
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp 50 55 60			192
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val 65 70 75 80			240
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu 85 90 95			288
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala 100 105 110			336
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser 115 120 125			384
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr 130 135 140			432
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser 145 150 155 160			480

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aaa aga agc caa ggt cga ata agg gaa ctt ttt tct caa gca gaa agt	528
Lys Arg Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	
165 170 175	
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg	576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	
180 185 190	
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta	624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	
195 200 205	
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa	672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	
210 215 220	
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac	720
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr	
225 230 235 240	
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga	768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	
245 250 255	
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa	816
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	
260 265 270	
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat	864
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	
275 280 285	
gtt cgg tta tac cca aaa ggg gtt aaa aca gaa cta aca aga gac att	912
Val Arg Leu Tyr Pro Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	
290 295 300	
tct acg gat cca att ttt gcc gtt aat act ctg tgg gaa tac gga cca	960
Ser Thr Asp Pro Ile Phe Ala Val Asn Thr Leu Trp Glu Tyr Gly Pro	
305 310 315 320	
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat	1008
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	
325 330 335	
tat tta cag ggg att gaa ttt cat acg cgt ctt cga cct ggt tac ttt	1056
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Arg Pro Gly Tyr Phe	
340 345 350	
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gca gaa act aga	1104
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Ala Glu Thr Arg	
355 360 365	
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat	1152
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp	
370 375 380	
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt	1200
Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val	
385 390 395 400	
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag	1248
Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys	
405 410 415	
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa	1296
Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln	
420 425 430	
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc	1344
Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly	
435 440 445	
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca	1392
His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr	
450 455 460	
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa	1440
Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu	
465 470 475 480	

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tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg	1488
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp	
485 490 495	
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att	1536
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile	
500 505 510	
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc	1584
Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser	
515 520 525	
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa	1632
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys	
530 535 540	
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca	1680
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala	
545 550 555 560	
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act	1728
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr	
565 570 575	
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc	1776
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile	
580 585 590	
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca	1824
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr	
595 600 605	
ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag	1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys	
610 615 620	
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc	1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile	
625 630 635 640	
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa	1959
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu	
645 650	

<210> SEQ ID NO 62

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 62

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	

Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr
130						135				140					
Val	Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Thr	Pro	Leu	Ser	Leu	Arg	Ser
145						150				155		160			
Lys	Arg	Ser	Gln	Gly	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser
		165						170		175					
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val
		180						185		190					
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu
195						200						205			
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu
210						215				220					
Asp	Val	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr
225						230				235		240			
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg
		245						250		255					
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu
		260						265		270					
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp
275						280						285			
Val	Arg	Leu	Tyr	Pro	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile
290						295				300					
Ser	Thr	Asp	Pro	Ile	Phe	Ala	Val	Asn	Thr	Leu	Trp	Glu	Tyr	Gly	Pro
305		310								315		320			
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp
		325						330		335					
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Arg	Pro	Gly	Tyr	Phe
		340						345		350					
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Ala	Glu	Thr	Arg
355						360						365			
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp
370						375				380					
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val
385		390								395		400			
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys
		405						410		415					
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln
		420						425		430					
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly
435						440						445			
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr
450						455				460					
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu
465		470								475		480			
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp
		485						490		495					
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile
		500						505		510					
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser
515						520						525			
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu			

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545	550	555	560	
Ala Leu Leu Gln Arg	Tyr Arg Val Arg	Ile Arg Tyr Ala Ser	Thr Thr	
565		570	575	
Asn Leu Arg Leu Phe	Val Gln Asn Ser	Asn Asn Asp Phe	Leu Val Ile	
580	585		590	
Tyr Ile Asn Lys Thr	Met Asn Lys Asp	Asp Asp Leu Thr	Tyr Gln Thr	
595	600	605		
Phe Asp Leu Ala Thr	Thr Asn Ser Asn	Met Gly Phe Ser	Gly Asp Lys	
610	615	620		
Asn Glu Leu Ile Ile	Gly Ala Glu Ser	Phe Val Ser Asn	Glu Lys Ile	
625	630	635	640	
Tyr Ile Asp Lys Ile	Glu Phe Ile Pro	Val Gln Leu		
645	650			
 <210> SEQ ID NO 63				
<211> LENGTH: 1959				
<212> TYPE: DNA				
<213> ORGANISM: Artificial sequence				
<220> FEATURE:				
<223> OTHER INFORMATION: Recombinant delta endotoxin				
<220> FEATURE:				
<221> NAME/KEY: CDS				
<222> LOCATION: (1)..(1956)				
 <400> SEQUENCE: 63				
atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct				48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro				
1 5 10 15				
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat				96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn				
20 25 30				
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg				144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met				
35 40 45				
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat				192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp				
50 55 60				
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta				240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val				
65 70 75 80				
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt				288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu				
85 90 95				
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca				336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala				
100 105 110				
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt				384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser				
115 120 125				
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat				432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr				
130 135 140				
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt				480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser				
145 150 155 160				
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt				528
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser				
165 170 175				
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg				576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val				

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180	185	190	
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu 195 200 205			624
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu 210 215 220			672
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr 225 230 235 240			720
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg 245 250 255			768
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu 260 265 270			816
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp 275 280 285			864
gtt cgg tta tac cca aaa ggg gtt aaa aca gaa cta aca aga gac att Val Arg Leu Tyr Pro Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile 290 295 300			912
ttt acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro 305 310 315 320			960
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp 325 330 335			1008
tat tta cag ggg att gaa ttt cat acg cgt ctt cga cct ggt tac ttt Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Arg Pro Gly Tyr Phe 340 345 350			1056
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg 355 360 365			1104
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp 370 375 380			1152
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 385 390 395 400			1200
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys 405 410 415			1248
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln 420 425 430			1296
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly 435 440 445			1344
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr 450 455 460			1392
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu 465 470 475 480			1440
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp 485 490 495			1488
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att			1536

Lys	Arg	Ser	Gln	Asp	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser
				165					170					175	
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val
			180					185					190		
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu
		195					200					205			
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu
		210				215					220				
Asp	Val	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr
225					230					235					240
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg
			245					250						255	
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu
			260					265					270		
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp
		275					280					285			
Val	Arg	Leu	Tyr	Pro	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile
		290				295					300				
Phe	Thr	Asp	Pro	Ile	Phe	Ser	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro
305					310					315					320
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp
				325					330					335	
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Arg	Pro	Gly	Tyr	Phe
			340					345					350		
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg
		355					360					365			
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp
		370				375					380				
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val
385					390					395					400
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys
			405					410						415	
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln
		420						425					430		
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly
		435					440					445			
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr
		450				455					460				
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu
465					470				475						480
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp
			485					490						495	
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile
			500					505					510		
Thr	Gln	Leu	Pro												

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580										585					590					
Tyr	Ile	Asn	Lys	Thr	Met	Asn	Lys	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr					
595										600					605					
Phe	Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys					
610										615					620					
Asn	Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile					
625										630					635					640
Tyr	Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu									
645										650										
<210> SEQ ID NO 65 <211> LENGTH: 1959 <212> TYPE: DNA <213> ORGANISM: Artificial sequence <220> FEATURE: <223> OTHER INFORMATION: Recombinant delta endotoxin <220> FEATURE: <221> NAME/KEY: CDS <222> LOCATION: (1)..(1956) <400> SEQUENCE: 65																				
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Met	Asn	Pro	Asn	Asn	Arg	Ser	Glu	His	Asp	Thr	Ile	Lys	Val	Thr	Pro					
1			5						10				15							
aac	agt	gaa	ttg	caa	act	aac	cat	aat	caa	tat	cct	tta	gct	gac	aat	96				
Asn	Ser	Glu	Leu	Gln	Thr	Asn	His	Asn	Gln	Tyr	Pro	Leu	Ala	Asp	Asn					
			20				25					30								
cca	aat	tca	aca	cta	gaa	gaa	tta	aat	tat	aaa	gaa	ttt	tta	aga	atg	144				
Pro	Asn	Ser	Thr	Leu	Glu	Glu	Leu	Asn	Tyr	Lys	Glu	Phe	Leu	Arg	Met					
			35				40					45								
act	gaa	gac	agt	tct	acg	gaa	gtg	cta	gac	aac	tct	aca	gta	aaa	gat	192				
Thr	Glu	Asp	Ser	Ser	Thr	Glu	Val	Leu	Asp	Asn	Ser	Thr	Val	Lys	Asp					
	50					55				60										
gca	gtt	ggg	aca	gga	att	tct	gtt	gta	ggg	cag	att	tta	ggt	gtt	gta	240				
Ala	Val	Gly	Thr	Gly	Ile	Ser	Val	Val	Gly	Gln	Ile	Leu	Gly	Val	Val					
65				70					75				80							
gga	gtt	cca	ttt	gct	ggg	gca	ctc	act	tca	ttt	tat	caa	tca	ttt	ctt	288				
Gly	Val	Pro	Phe	Ala	Gly	Ala	Leu	Thr	Ser	Phe	Tyr	Gln	Ser	Phe	Leu					
			85					90					95							
aac	act	ata	tgg	cca	agt	gat	gct	gac	cca	tgg	aag	gct	ttt	atg	gca	336				
Asn	Thr	Ile	Trp	Pro	Ser	Asp	Ala	Asp	Pro	Trp	Lys	Ala	Phe	Met	Ala					
			100					105					110							
caa	gtt	gaa	gta	ctg	ata	gat	aag	aaa	ata	gag	gag	tat	gct	aaa	agt	384				
Gln	Val	Glu	Val	Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser					
		115					120					125								
aaa	gct	ctt	gca	gag	tta	cag	ggt	ctt	caa	aat	aat	ttc	gaa	gat	tat	432				
Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr					
	130				135					140										
gtt	aat	gcg	tta	aat	tcc	tgg	aag	aaa	aca	cct	tta	agt	ttg	cga	agt	480				
Val	Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Thr	Pro	Leu	Ser	Leu	Arg	Ser					
145				150					155				160							
aaa	aga	agc	caa	ggt	cga	ata	agg	gaa	ctt	ttt	tct	caa	gca	gaa	agt	528				
Lys	Arg	Ser	Gln	Gly	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser					
			165					170					175							
cat	ttt	cgt	aat	tcc	atg	ccg	tca	ttt	gca	gtt	tcc	aaa	ttc	gaa	gtg	576				
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val					
		180					185					190								
ctg	ttt	cta	cca	aca	tat	gca	caa	gct	gca	aat	aca	cat	tta	ttg	cta	624				
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu					
		195				200						205								

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tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	672
210 215 220	
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr	720
225 230 235 240	
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	768
245 250 255	
ggg tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	816
260 265 270	
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	864
275 280 285	
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	912
290 295 300	
ttt acg gat cca att ttt tta ctt aat act ctt cag gag tat gga cca Phe Thr Asp Pro Ile Phe Leu Leu Asn Thr Leu Gln Glu Tyr Gly Pro	960
305 310 315 320	
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	1008
325 330 335	
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe	1056
340 345 350	
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg	1104
355 360 365	
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp	1152
370 375 380	
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val	1200
385 390 395 400	
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys	1248
405 410 415	
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln	1296
420 425 430	
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly	1344
435 440 445	
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr	1392
450 455 460	
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu	1440
465 470 475 480	
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp	1488
485 490 495	
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile	1536
500 505 510	
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser	1584
515 520 525	

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att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa	1632
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys	
530 535 540	
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca	1680
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala	
545 550 555 560	
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act	1728
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr	
565 570 575	
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc	1776
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile	
580 585 590	
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca	1824
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr	
595 600 605	
ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat aag	1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys	
610 615 620	
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc	1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile	
625 630 635 640	
tat ata gat aag ata gaa ttt atc cca gta caa ttg taa	1959
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu	
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<210> SEQ ID NO 66

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 66

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Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser	
145 150 155 160	
Lys Arg Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	
165 170 175	
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	
180 185 190	

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Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu
	195						200					205			
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu
	210					215					220				
Asp	Val	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr
225					230					235					240
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg
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Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu
		260					265						270		
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp
	275					280						285			
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile
	290				295						300				
Phe	Thr	Asp	Pro	Ile	Phe	Leu	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro
305				310						315					320
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp
			325						330					335	
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe
		340						345					350		
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg
	355						360					365			
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp
	370					375					380				
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val
385				390						395					400
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys
			405					410						415	
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln
		420					425					430			
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly
	435					440						445			
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr
	450				455						460				
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu
465				470						475					480
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp
		485						490						495	
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile
		500						505					510		
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser
	515					520						525			
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys
	530				535						540				
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala
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Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr
			565					570						575	
Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile
		580						585					590		
Tyr	Ile	Asn	Lys	Thr	Met	Asn	Lys	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr
	595					600						605			
Phe	Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys

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610	615	620	
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile			
625	630	635	640
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu			
	645	650	
 <210> SEQ ID NO 67			
<211> LENGTH: 1959			
<212> TYPE: DNA			
<213> ORGANISM: Artificial sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Recombinant delta endotoxin			
<220> FEATURE:			
<221> NAME/KEY: CDS			
<222> LOCATION: (1)..(1956)			
 <400> SEQUENCE: 67			
atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct			48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro			
1	5	10	15
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat			96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn			
	20	25	30
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg			144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met			
	35	40	45
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat			192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp			
	50	55	60
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta			240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val			
	65	70	80
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt			288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu			
	85	90	95
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca			336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala			
	100	105	110
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt			384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser			
	115	120	125
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat			432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr			
	130	135	140
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt			480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser			
	145	150	155
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt			528
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser			
	165	170	175
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg			576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val			
	180	185	190
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta			624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu			
	195	200	205
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa			672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu			
	210	215	220
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac			720
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr			

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225	230	235	240	
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga				768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	245	250	255	
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa				816
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	260	265	270	
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat				864
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	275	280	285	
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att				912
Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	290	295	300	
ttt acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca				960
Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro	305	310	315	320
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat				1008
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	325	330	335	
tat tta cag ggg att gaa ttt cat acg cgt ctt cga cct ggt tac ttt				1056
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Arg Pro Gly Tyr Phe	340	345	350	
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga				1104
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg	355	360	365	
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat				1152
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp	370	375	380	
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt				1200
Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val	385	390	395	400
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag				1248
Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys	405	410	415	
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa				1296
Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln	420	425	430	
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc				1344
Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly	435	440	445	
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca				1392
His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr	450	455	460	
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa				1440
Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu	465	470	475	480
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg				1488
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp	485	490	495	
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att				1536
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile	500	505	510	
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc				1584
Thr Gln Leu Pro Val Val Lys Ala Tyr Ser His Ala Leu Ser Ser Gly Ala Ser	515	520	525	
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa				1632
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys	530	535	540	
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca				1680

Asp 225	Val	Ala	Glu	Phe	Tyr 230	His	Arg	Gln	Leu	Lys 235	Leu	Thr	Gln	Gln	Tyr 240
Thr	Asp	His	Cys	Val 245	Asn	Trp	Tyr	Asn	Val 250	Gly	Leu	Asn	Gly	Leu 255	Arg
Gly	Ser	Thr	Tyr 260	Asp	Ala	Trp	Val	Lys 265	Phe	Asn	Arg	Phe	Arg 270	Arg	Glu
Met	Thr	Leu 275	Thr	Val	Leu	Asp	Leu 280	Ile	Val	Leu	Phe	Pro 285	Phe	Tyr	Asp
Ile	Arg 290	Leu	Tyr	Ser	Lys 295	Gly	Val	Lys	Thr	Glu 300	Leu	Thr	Arg	Asp	Ile
Phe 305	Thr	Asp	Pro	Ile 310	Phe	Ser	Leu	Asn	Thr	Leu 315	Gln	Glu	Tyr	Gly	Pro 320
Thr	Phe	Leu	Ser 325	Ile	Glu	Asn	Ser	Ile	Arg 330	Lys	Pro	His	Leu	Phe 335	Asp
Tyr	Leu	Gln	Gly 340	Ile	Glu	Phe	His	Thr 345	Arg	Leu	Arg	Pro	Gly 350	Tyr	Phe
Gly	Lys	Asp 355	Ser	Phe	Asn	Tyr	Trp 360	Ser	Gly	Asn	Tyr	Val 365	Glu	Thr	Arg
Pro 370	Ser	Ile	Gly	Ser	Ser	Lys 375	Thr	Ile	Thr	Ser	Pro 380	Phe	Tyr	Gly	Asp
Lys 385	Ser	Thr	Glu	Pro	Val 390	Gln	Lys	Leu	Ser	Phe 395	Asp	Gly	Gln	Lys	Val 400
Tyr	Arg	Thr	Ile 405	Ala	Asn	Thr	Asp	Val	Ala 410	Ala	Trp	Pro	Asn	Gly 415	Lys
Val	Tyr	Leu	Gly 420	Val	Thr	Lys	Val	Asp 425	Phe	Ser	Gln	Tyr	Asp 430	Asp	Gln
Lys	Asn	Glu 435	Thr	Ser	Thr	Gln	Thr 440	Tyr	Asp	Ser	Lys	Arg 445	Asn	Asn	Gly
His 450	Val	Ser	Ala	Gln	Asp 455	Ser	Ile	Asp	Gln	Leu	Pro 460	Pro	Glu	Thr	Thr
Asp 465	Glu	Pro	Leu	Glu	Lys 470	Ala	Tyr	Ser	His	Gln 475	Leu	Asn	Tyr	Ala	Glu 480
Cys	Phe	Leu	Met 485	Gln	Asp	Arg	Arg	Gly	Thr 490	Ile	Pro	Phe	Phe	Thr 495	Trp
Thr	His	Arg 500	Ser	Val	Asp	Phe	Phe	Asn 505	Thr	Ile	Asp	Ala	Glu 510	Lys	Ile
Thr	Gln	Leu 515	Pro	Val	Val	Lys	Ala 520	Tyr	Ala	Leu	Ser	Ser 525	Gly	Ala	Ser
Ile 530	Ile	Glu	Gly	Pro	Gly 535	Phe	Thr	Gly	Gly	Asn 540	Leu	Leu	Phe	Leu	Lys
Glu 545	Ser	Ser	Asn	Ser	Ile 550	Ala	Lys	Phe	Lys	Val 555	Thr	Leu	Asn	Ser	Ala 560
Ala	Leu	Leu	Gln 565	Arg	Tyr	Arg	Val	Arg	Ile 570	Arg	Tyr	Ala	Ser	Thr 575	Thr
Asn	Leu	Arg 580	Leu	Phe	Val	Gln	Asn	Ser 585	Asn	Asn	Asp	Phe	Leu 590	Val	Ile
Tyr	Ile 595	Asn	Lys	Thr	Met	Asn	Lys 600	Asp	Asp	Asp	Leu	Thr 605	Tyr	Gln	Thr
Phe 610	Asp	Leu	Ala	Thr	Thr	Asn 615	Ser	Asn	Met	Gly	Phe 620	Ser	Gly	Asp	Lys
Asn 625	Glu	Leu	Ile	Ile	Gly 630	Ala	Glu	Ser	Phe	Val 635	Ser	Asn	Glu	Lys	Ile 640
Tyr	Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu				

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645	650	
<210> SEQ ID NO 69		
<211> LENGTH: 1482		
<212> TYPE: DNA		
<213> ORGANISM: Artificial sequence		
<220> FEATURE:		
<223> OTHER INFORMATION: Recombinant delta endotoxin		
<220> FEATURE:		
<221> NAME/KEY: CDS		
<222> LOCATION: (1)..(1479)		
<400> SEQUENCE: 69		
agt aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa		48
Ser Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu		
1 5 10 15		
agt cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa		96
Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu		
20 25 30		
gtg ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg		144
Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu		
35 40 45		
cta tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca		192
Leu Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser		
50 55 60		
gaa gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa		240
Glu Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln		
65 70 75 80		
tac act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta		288
Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu		
85 90 95		
aga ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga		336
Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg		
100 105 110		
gaa atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat		384
Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr		
115 120 125		
gat att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac		432
Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp		
130 135 140		
att ttt acg gat cca att ttt tca ctt aat act ctt cag gag tat gga		480
Ile Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly		
145 150 155 160		
cca act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt		528
Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe		
165 170 175		
gat tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac		576
Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr		
180 185 190		
ttt ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act		624
Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr		
195 200 205		
aga cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga		672
Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly		
210 215 220		
gat aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa		720
Asp Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys		
225 230 235 240		
gtt tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt		768
Val Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly		
245 250 255		

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aag gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat Lys Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp 260 265 270	816
caa aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn 275 280 285	864
ggc cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca Gly His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr 290 295 300	912
aca gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala 305 310 315 320	960
gaa tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act Glu Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr 325 330 335	1008
tggt aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag Trp Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys 340 345 350	1056
att act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct Ile Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala 355 360 365	1104
tcc att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta Ser Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu 370 375 380	1152
aaa gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser 385 390 395 400	1200
gca gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr 405 410 415	1248
act aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc Thr Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val 420 425 430	1296
atc tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln 435 440 445	1344
aca ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp 450 455 460	1392
aag aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys 465 470 475 480	1440
atc tat ata gat aag ata gaa ttt atc cca gta caa ttg taa Ile Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu 485 490	1482

<210> SEQ ID NO 70

<211> LENGTH: 493

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 70

Ser Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu 1 5 10 15
--

Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu 20 25 30

Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu 35 40 45

Leu 50	Leu	Lys	Asp	Ala	Gln	Val 55	Phe	Gly	Glu	Glu	Trp 60	Gly	Tyr	Ser	Ser
Glu 65	Asp	Val	Ala	Glu	Phe 70	Tyr	His	Arg	Gln	Leu 75	Lys	Leu	Thr	Gln	Gln 80
Tyr	Thr	Asp	His	Cys 85	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly 95	Leu
Arg	Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val 105	Lys	Phe	Asn	Arg	Phe 110	Arg	Arg
Glu	Met	Thr	Leu	Thr	Val	Leu	Asp 120	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr
Asp	Ile	Arg	Leu	Tyr	Ser	Lys 135	Gly	Val	Lys	Thr	Glu 140	Leu	Thr	Arg	Asp
Ile 145	Phe	Thr	Asp	Pro	Ile 150	Phe	Ser	Leu	Asn	Thr 155	Leu	Gln	Glu	Tyr	Gly 160
Pro	Thr	Phe	Leu	Ser 165	Ile	Glu	Asn	Ser	Ile 170	Arg	Lys	Pro	His	Leu 175	Phe
Asp	Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His 185	Thr	Arg	Leu	Gln	Pro	Gly	Tyr
Phe	Gly	Lys 195	Asp	Ser	Phe	Asn	Tyr 200	Trp	Ser	Gly	Asn	Tyr 205	Val	Glu	Thr
Arg	Pro	Ser	Ile	Gly	Ser	Ser 215	Lys	Thr	Ile	Thr	Ser 220	Pro	Phe	Tyr	Gly
Asp 225	Lys	Ser	Thr	Glu	Pro 230	Val	Gln	Lys	Leu	Ser 235	Phe	Asp	Gly	Gln	Lys 240
Val	Tyr	Arg	Thr	Ile 245	Ala	Asn	Thr	Asp	Val 250	Ala	Ala	Trp	Pro	Asn	Gly 255
Lys	Val	Tyr	Leu	Gly	Val	Thr	Lys	Val 265	Asp	Phe	Ser	Gln	Tyr 270	Asp	Asp
Gln	Lys	Asn 275	Glu	Thr	Ser	Thr	Gln 280	Thr	Tyr	Asp	Ser	Lys 285	Arg	Asn	Asn
Gly	His 290	Val	Ser	Ala	Gln	Asp 295	Ser	Ile	Asp	Gln 300	Leu	Pro	Pro	Glu	Thr
Thr 305	Asp	Glu	Pro	Leu	Glu 310	Lys	Ala	Tyr	Ser	His 315	Gln	Leu	Asn	Tyr	Ala 320
Glu	Cys	Phe	Leu	Met 325	Gln	Asp	Arg	Arg	Gly 330	Thr	Ile	Pro	Phe	Phe 335	Thr
Trp	Thr	His 340	Arg	Ser	Val	Asp	Phe 345	Phe	Asn	Thr	Ile	Asp 350	Ala	Glu	Lys
Ile	Thr	Gln 355	Leu	Pro	Val	Val	Lys 360	Ala	Tyr	Ala	Leu	Ser 365	Ser	Gly	Ala
Ser 370	Ile	Ile	Glu	Gly	Pro	Gly 375	Phe	Thr	Gly	Gly	Asn 380	Leu	Leu	Phe	Leu
Lys 385	Glu	Ser	Ser	Asn	Ser 390	Ile	Ala	Lys	Phe	Lys 395	Val	Thr	Leu	Asn	Ser 400
Ala	Ala	Leu	Leu	Gln 405	Arg	Tyr	Arg	Val	Arg 410	Ile	Arg	Tyr	Ala	Ser 415	Thr
Thr	Asn	Leu	Arg 420	Leu	Phe	Val	Gln	Asn 425	Ser	Asn	Asn	Asp 430	Phe	Leu	Val
Ile	Tyr	Ile 435	Asn	Lys	Thr	Met	Asn 440	Lys	Asp	Asp	Asp	Leu 445	Thr	Tyr	Gln
Thr 450	Phe	Asp	Leu	Ala	Thr	Thr 455	Asn	Ser	Asn	Met	Gly 460	Phe	Ser	Gly	Asp
Lys	Asn	Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys

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465	470	475	480
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Ile Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 485 490

<210> SEQ ID NO 71
 <211> LENGTH: 23
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Oligonucleotide

<400> SEQUENCE: 71

agacaactct acagtaaaag atg 23

<210> SEQ ID NO 72
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Oligonucleotide

<400> SEQUENCE: 72

ggtaattggt caatagaatc 20

<210> SEQ ID NO 73
 <211> LENGTH: 39
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Oligonucleotide
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (21)..(23)
 <223> OTHER INFORMATION: N = A, T, G, C (25% each)

<400> SEQUENCE: 73

cagaagatgt tgctgaattc nnncatagac aattaaaac 39

<210> SEQ ID NO 74
 <211> LENGTH: 34
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Oligonucleotide
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (19)..(21)
 <223> OTHER INFORMATION: N = A, T, G, C (25% each)

<400> SEQUENCE: 74

gatgttgctg aattctatnn nagacaatta aaac 34

<210> SEQ ID NO 75
 <211> LENGTH: 33
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Oligonucleotide
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (17)..(17)
 <223> OTHER INFORMATION: N = A, T, C (16% each); G (52%)
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (18)..(18)
 <223> OTHER INFORMATION: N = T, G, C (10% each); A (70%)
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (19)..(19)

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<223> OTHER INFORMATION: N = A, T, G, C (25% each)

<400> SEQUENCE: 75

ccccattttat gatattnnnt tataactcaaa agg

33

<210> SEQ ID NO 76

<211> LENGTH: 64

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Oligonucleotide

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (24)..(24)

<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (25)..(25)

<223> OTHER INFORMATION: N = A, T, G (6% each); C (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (26)..(26)

<223> OTHER INFORMATION: N = A, T, G, C (25% each)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (27)..(28)

<223> OTHER INFORMATION: N = A, T, G (6% each); C (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (29)..(29)

<223> OTHER INFORMATION: N = A, T, G, C (25% each)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (30)..(30)

<223> OTHER INFORMATION: N = A, T, G (6% each); C (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (31)..(31)

<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (32)..(32)

<223> OTHER INFORMATION: N = A, T, G, C (25%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (33)..(33)

<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (34)..(34)

<223> OTHER INFORMATION: N = A, T, G (6% each) ; C (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (35)..(35)

<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (36)..(36)

<223> OTHER INFORMATION: N = A, T, G (6% each) ; C (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (37)..(37)

<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (38)..(38)

<223> OTHER INFORMATION: N = A, T, G, C (25% each)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (39)..(39)

<223> OTHER INFORMATION: N = A, T, G (6% each); C (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (40)..(40)

<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

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<222> LOCATION: (41)..(41)
<223> OTHER INFORMATION: N = A, T, G, C (25% each)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (42)..(42)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (43)..(43)
<223> OTHER INFORMATION: N = A, T, G (6% each); C (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (44)..(44)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)

<400> SEQUENCE: 76

agctatgctg gtctcggaag aaannnnnnn nnnnnnnnnn nnnnaaaaga agccaagatc 60
gaat 64

<210> SEQ ID NO 77
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

<400> SEQUENCE: 77

ggtcacctag gtctctcttc caggaattta acgcattaac 40

<210> SEQ ID NO 78
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(23)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (24)..(24)
<223> OTHER INFORMATION: N = A, T, G (1% each); C (97%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (25)..(25)
<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (26)..(26)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (27)..(27)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (28)..(28)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (29)..(29)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (30)..(30)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(31)

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<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (32)..(33)
<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (34)..(34)
<223> OTHER INFORMATION: N = A, T, G (15% each); C (55%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (35)..(36)
<223> OTHER INFORMATION: N = A, G, C (15% each); T (55%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (37)..(37)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (38)..(38)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (39)..(39)
<223> OTHER INFORMATION: N = A, T, G (1% each); C (97%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (40)..(40)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (41)..(41)
<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (42)..(42)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (43)..(44)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (45)..(45)
<223> OTHER INFORMATION: N = A, T, G, C (25% each)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (46)..(48)
<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)

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<400> SEQUENCE: 78

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agctatgctg gtctccatt tnnnnnnnnn nnnnnnnnnn nnnnnnnngt taaaacagaa      60

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ctaac                                                                    65

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<210> SEQ ID NO 79
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

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<400> SEQUENCE: 79

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atccagtggg gtctcaaatg ggaaaagtac aattag                                36

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<210> SEQ ID NO 80
<211> LENGTH: 63
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(23)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)

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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (24)..(26)
<223> OTHER INFORMATION: N = A, T, G (6% each); C (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (27)..(27)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (28)..(28)
<223> OTHER INFORMATION: N = A, T, G (6% each); C (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (29)..(30)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(31)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (32)..(32)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (33)..(33)
<223> OTHER INFORMATION: N = A, T, G (6% each); C (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (34)..(34)
<223> OTHER INFORMATION: N = A, T, G (6% each); C (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (35)..(35)
<223> OTHER INFORMATION: N = A, T, G (6% each); C (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (36)..(36)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (37)..(37)
<223> OTHER INFORMATION: N = A, T, G (6% each); C (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (38)..(38)
<223> OTHER INFORMATION: N = A, T, G (6% each); C (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (39)..(39)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (40)..(40)
<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (41)..(41)
<223> OTHER INFORMATION: N = A, C (8% each); T (1%); G (83%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (42)..(42)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (43)..(43)
<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (44)..(44)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (45)..(45)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (46)..(46)

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<223> OTHER INFORMATION: N = A, T, G (1% each); C (97%)

<400> SEQUENCE: 80

cattttttacg gatccaattt tttnnnnnnnn nnnnnnnnnn nnnnnnggac caactttttt 60

gag 63

<210> SEQ ID NO 81

<211> LENGTH: 62

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Oligonucleotide

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (28)..(28)

<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (29)..(29)

<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (30)..(30)

<223> OTHER INFORMATION: N = A, T, G (1% each); C (97%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (31)..(31)

<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (34)..(35)

<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (36)..(36)

<223> OTHER INFORMATION: N = A, T, G, C (25% each)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (37)..(37)

<223> OTHER INFORMATION: N = A (82%); T (2%); G, C (8% each)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (38)..(39)

<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (40)..(40)

<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (41)..(41)

<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (42)..(42)

<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)

<400> SEQUENCE: 81

gaatttcata cgcgtcttca acctgggttnn nnnnnnnnnn nntctttcaa ttattggtct 60

gg 62

<210> SEQ ID NO 82

<211> LENGTH: 73

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Oligonucleotide

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (41)..(41)

<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)

<220> FEATURE:

<221> NAME/KEY: misc_feature

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<222> LOCATION: (42)..(43)
<223> OTHER INFORMATION: N = A (0%); T,C (9% each); G (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (44)..(45)
<223> OTHER INFORMATION: N = A, T, G (6% each); C (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (46)..(46)
<223> OTHER INFORMATION: N = A, T, G, C (25% each)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (47)..(48)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (49)..(49)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (50)..(51)
<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (52)..(52)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (53)..(54)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (55)..(55)
<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)

<400> SEQUENCE: 82

aaaagtttat cgaactatag ctaatacaga cgtagcggct nnnnnnnnnn nnnngtata 60
tttagtgttt acg 73

<210> SEQ ID NO 83
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

<400> SEQUENCE: 83

ggagttccat ttgtggggc 20

<210> SEQ ID NO 84
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

<400> SEQUENCE: 84

atctccataa aatgggg 17

<210> SEQ ID NO 85
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

<400> SEQUENCE: 85

gcgaagtaaa agaagccaag gtcgaataag gg 32

<210> SEQ ID NO 86
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<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

<400> SEQUENCE: 86

cctttaagtt tgcgaaatcc acacagccaa ggtcgaataa ggg 43

<210> SEQ ID NO 87
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

<400> SEQUENCE: 87

cccattttat gatgttcggt tataacccaaa agggg 35

<210> SEQ ID NO 88
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

<400> SEQUENCE: 88

ggccaagtga agacccatgg aaggc 25

<210> SEQ ID NO 89
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

<400> SEQUENCE: 89

gcagtttccg gattcgaagt gc 22

<210> SEQ ID NO 90
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

<400> SEQUENCE: 90

ccgctacgtc tgtatta 17

<210> SEQ ID NO 91
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

<400> SEQUENCE: 91

ataatggaag cacctga 17

<210> SEQ ID NO 92
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide
<220> FEATURE:
<221> NAME/KEY: misc_feature

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<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(23)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (24)..(24)
<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (25)..(25)
<223> OTHER INFORMATION: N = A, T, G (6% each); C (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (26)..(26)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (27)..(28)
<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (29)..(29)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (30)..(31)
<223> OTHER INFORMATION: N = A, T, G (6% each); C (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (32)..(32)
<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (33)..(33)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (34)..(34)
<223> OTHER INFORMATION: N = A, T, G (6% each); C (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (35)..(35)
<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (36)..(36)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (37)..(38)
<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (39)..(39)
<223> OTHER INFORMATION: N = A, T, G, C (25% each)

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<400> SEQUENCE: 92

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agctatgctg gtctcttctt annnnnnnnn nnnnnnnnna caattccatt ttttacttgg 60

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<210> SEQ ID NO 93
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

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<400> SEQUENCE: 93

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atccagttgg gtctctaaga aacaaaccgc gtaattaagc 40

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<210> SEQ ID NO 94
<211> LENGTH: 20
<212> TYPE: DNA

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide
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<400> SEQUENCE: 94
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cctcaagggt tataacatcc
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20

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<210> SEQ ID NO 95
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (22)..(23)
<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (24)..(24)
<223> OTHER INFORMATION: N = A, T, G, C (25% each)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (25)..(25)
<223> OTHER INFORMATION: N = A, G (8% each); T (2%); C (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (26)..(27)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (28)..(28)
<223> OTHER INFORMATION: N = A (82%); T (2%); G,C (8% each)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (29)..(30)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(31)
<223> OTHER INFORMATION: N = A, T, C (6% each); G (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (32)..(32)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (33)..(33)
<223> OTHER INFORMATION: N = A, T, G, C (25% each)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (34)..(34)
<223> OTHER INFORMATION: N = A, G, C (6% each); T (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (35)..(35)
<223> OTHER INFORMATION: N = T, G, C (6% each); A (82%)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (36)..(36)
<223> OTHER INFORMATION: N = A, G, C (1% each); T (97%)
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<400> SEQUENCE: 95
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gtacaaaagc taagctttnn nnnnnnnnnn nnnnnncgaa ctatagctaa tacag
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<210> SEQ ID NO 96
 <211> LENGTH: 7
 <212> TYPE: PRT
 <213> ORGANISM: *Bacillus thuringiensis*

<400> SEQUENCE: 96

Ser Lys Arg Ser Gln Asp Arg
 1 5

<210> SEQ ID NO 97
 <211> LENGTH: 1959
 <212> TYPE: DNA
 <213> ORGANISM: *Bacillus thuringiensis*
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1956)

<400> SEQUENCE: 97

atg aat cca aac aat cga agt gaa cat gat acg ata aag gtt aca cct	48
Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca	336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt	384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat	432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt	480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser	
145 150 155 160	
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt	528
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	
165 170 175	
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg	576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	
180 185 190	
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta	624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	
195 200 205	
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa	672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	
210 215 220	

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gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac	720
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr	
225 230 235 240	
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga	768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	
245 250 255	
ggg tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa	816
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	
260 265 270	
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat	864
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	
275 280 285	
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att	912
Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	
290 295 300	
ttt acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca	960
Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro	
305 310 315 320	
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat	1008
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	
325 330 335	
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt	1056
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe	
340 345 350	
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga	1104
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg	
355 360 365	
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat	1152
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp	
370 375 380	
aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt	1200
Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val	
385 390 395 400	
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag	1248
Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys	
405 410 415	
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa	1296
Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln	
420 425 430	
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc	1344
Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly	
435 440 445	
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca	1392
His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr	
450 455 460	
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa	1440
Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu	
465 470 475 480	
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg	1488
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp	
485 490 495	
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att	1536
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile	
500 505 510	
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc	1584
Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser	
515 520 525	
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa	1632
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys	
530 535 540	

Met	Asn	Pro	Asn	Asn	Arg	Ser	Glu	His	Asp	Thr	Ile	Lys	Val	Thr	Pro
1				5					10					15	
Asn	Ser	Glu	Leu	Gln	Thr	Asn	His	Asn	Gln	Tyr	Pro	Leu	Ala	Asp	Asn
			20					25					30		
Pro	Asn	Ser	Thr	Leu	Glu	Glu	Leu	Asn	Tyr	Lys	Glu	Phe	Leu	Arg	Met
		35					40					45			
Thr	Glu	Asp	Ser	Ser	Thr	Glu	Val	Leu	Asp	Asn	Ser	Thr	Val	Lys	Asp
	50					55					60				
Ala	Val	Gly	Thr	Gly	Ile	Ser	Val	Val	Gly	Gln	Ile	Leu	Gly	Val	Val
65					70				75						80
Gly	Val	Pro	Phe	Ala	Gly	Ala	Leu	Thr	Ser	Phe	Tyr	Gln	Ser	Phe	Leu
			85						90					95	
Asn	Thr	Ile	Trp	Pro	Ser	Asp	Ala	Asp	Pro	Trp	Lys	Ala	Phe	Met	Ala
			100					105					110		
Gln	Val	Glu	Val	Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser
		115					120					125			
Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr
	130					135					140				
Val	Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Thr	Pro	Leu	Ser	Leu	Arg	Ser
145					150					155					160
Lys	Arg	Ser	Gln	Asp	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser
			165					170						175	
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val
			180					185					190		
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu
		195					200					205			
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu
	210				215						220				

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Asp	Val	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr
225					230					235					240
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg
			245						250					255	
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu
			260					265					270		
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp
		275					280					285			
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile
	290					295					300				
Phe	Thr	Asp	Pro	Ile	Phe	Ser	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro
305					310					315					320
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp
				325					330					335	
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe
			340					345					350		
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg
		355					360					365			
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp
		370				375					380				
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val
385					390					395					400
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys
				405					410					415	
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln
		420						425					430		
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly
		435					440					445			
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr
	450					455					460				
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu
465					470					475					480
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp
				485					490					495	
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile
			500					505					510		
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser
		515					520					525			
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys
	530					535					540				
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala
545					550					555					560
Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr
				565					570					575	
Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile
			580					585					590		
Tyr	Ile	Asn	Lys	Thr	Met	Asn	Lys	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr
		595					600					605			
Phe	Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys
	610					615					620				
Asn	Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile
625					630					635					640
Tyr	Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu				

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645	650	
<210> SEQ ID NO 99		
<211> LENGTH: 2000		
<212> TYPE: DNA		
<213> ORGANISM: Artificial sequence		
<220> FEATURE:		
<223> OTHER INFORMATION: Recombinant delta endotoxin		
<400> SEQUENCE: 99		
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tctgagttgc aaactaatca caaccagtac ccattggctg acaatcctaa cagtactctt	120	
gaggaaactta actacaagga gtttctccgg atgaccgaag atagctccac tgaggttctc	180	
gataactcta cagtgaagga cgctgttgga actggcatta gcgttggtgg acagattctt	240	
ggagtgggtg gtgttcatt cgctggagct ttgaccagct tctaccagtc ctttctcaac	300	
accatctggc cttcagatgc tgatccctgg aaggctttca tggcccaagt ggaagtcttg	360	
atcgataaga agatcgaaga gtatgccaaag tctaaagcct tggctgagtt gcaaggtttg	420	
cagaacaact tcgaggatta cgtcaacgca ctcaacagct ggaagaaaac tcccttgagt	480	
ctcaggtcta agcgttccca ggaccgtatt cgtgaacttt tcagccaagc cgaatccac	540	
ttcagaaaact ccatgcctag ctttgccgtt tctaagttcg aggtgctctt cttgccaaca	600	
tacgcacaag ctgccaacac tcattctctg cttctcaaag acgctcaggt gtttggtgag	660	
gaatggggtt actccagtga agatgttgcc gagttctacc gtaggcagct caagttgact	720	
caacagtaca cagaccactg cgtcaactgg tacaacgttg ggctcaatgg tcttagagga	780	
tctacctacg acgcatgggt gaagttcaac aggtttcgta gagagatgac cttgactgtg	840	
ctcgatctta tcgttctctt tccattctac gacattcgtc tttactccaa aggcgttaag	900	
acagagctga ccagagacat cttcaccgat cccatcttcc tacttacgac cctgcagaaa	960	
tacgggtccaa cttttctctc cattgagaac agcatcagga agcctcacct cttcgactat	1020	
ctgcaaggca ttgagtttca caccaggttg caacctggtt acttcggtaa ggattccttc	1080	
aactactgga gcggaacta cgttgaaacc agaccatcca tcggatctag caagaccatc	1140	
acttctccat tctacggtga caagagcact gagccagtgc agaagttgag cttcgatggg	1200	
cagaaggtgt atagaacat cgccaatacc gatgttgag cttggcctaa tggcaaggtc	1260	
taccttgag ttactaaagt ggacttctcc caatacagac atcagaagaa cgagacatct	1320	
actcaaacct acgatagtaa gaggaacaat ggccatgttt ccgcacaaga ctccattgac	1380	
caacttccac ctgaaaccac tgatgaacca ttggagaagg cttacagtca ccaacttaac	1440	
tacgccgaat gttttctcat gcaagacagg cgtggcacca ttccgttctt tacatggact	1500	
cacaggtctg tcgacttctt taacactatc gacgtgaga agattacca acttcccgtg	1560	
gtcaaggctt atgccttgtc cagcggagct tccatcattg aaggtccagg cttcacgggt	1620	
ggcaacttgc ttttctctaa ggagtccagc aactccatcg ccaagttcaa agtgacactt	1680	
aactcagcag ctttgctcca acgttacagg gttcgtatca gatacgcaag cactaccaat	1740	
cttcgcctct ttgtccagaa cagcaacaat gatttccttg tcattctacat caacaagact	1800	
atgaacaaag acgatgacct cacctaccaa acattcgatc ttgccactac caatagtaac	1860	
atgggattct ctggtgacaa gaacgagctg atcataggtg ctgagagctt tgtctcta	1920	
gagaagattt acatagacaa gatcgagttc attccagttc aactctaata gatcccccg	1980	
gctgcaggaa ttcgatatca	2000	

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<210> SEQ ID NO 100
<211> LENGTH: 653
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 100

Met Ala Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr
 1             5             10            15

Pro Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp
 20            25            30

Asn Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg
 35            40            45

Met Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys
 50            55            60

Asp Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val
 65            70            75            80

Val Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe
 85            90            95

Leu Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met
100           105           110

Ala Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys
115           120           125

Ser Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp
130           135           140

Tyr Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg
145           150           155           160

Ser Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu
165           170           175

Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu
180           185           190

Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu
195           200           205

Leu Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser
210           215           220

Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln Gln
225           230           235           240

Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu
245           250           255

Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg
260           265           270

Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr
275           280           285

Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp
290           295           300

Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr Gly
305           310           315           320

Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe
325           330           335

Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr
340           345           350

Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr
355           360           365

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Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly
 370 375 380
 Asp Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys
 385 390 395 400
 Val Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly
 405 410 415
 Lys Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp
 420 425 430
 Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn
 435 440 445
 Gly His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr
 450 455 460
 Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala
 465 470 475 480
 Glu Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr
 485 490 495
 Trp Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys
 500 505 510
 Ile Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala
 515 520 525
 Ser Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu
 530 535 540
 Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser
 545 550 555 560
 Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr
 565 570 575
 Thr Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val
 580 585 590
 Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln
 595 600 605
 Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp
 610 615 620
 Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys
 625 630 635 640
 Ile Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> SEQ ID NO 101

<211> LENGTH: 2050

<212> TYPE: DNA

<213> ORGANISM: *Bacillus thuringiensis*

<400> SEQUENCE: 101

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aatcacaacc agtaccattt ggctgacaat cctaacagta ctcttgagga acttaactac    180
aaggagtttc tccggatgac cgaagatagc tccactgagg ttctcgataa ctctacagtg    240
aaggacgctg ttggaactgg cattagcgtt gtgggacaga ttcttgaggt ggttggtgtt    300
ccattcgctg gagctttgac cagcttctac cagtcccttc tcaacaccaa ctggccttca    360
gatgctgctc cctggaaggc ttctatggcc caagtgaag tcttgatcga taagaagatc    420
gaagagtatg ccaagtctaa agccttgctt gagttgcaag gtttcagaaa caacttcgag    480

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gattacgtca acgcaactcaa cagctggaag aaaactccct tgagtctcag gtctaagcgt	540
tcccaggacc gtattcgtga acttttcagc caagccgaat cccacttcag aaactccatg	600
cctagctttg cggtttctaa gttcgagggtg ctcttcttgc caacatacgc acaagctgcc	660
aacactcatc tcttgcttct caaagacgct caggtgtttg gtgaggaatg gggttactcc	720
agtgaagatg ttgccgagtt ctaccatagg cagctcaagt tgactcaaca gtacacagac	780
cactgcgtca actggtacaa cgttgggctc aatggtctta gaggatctac ctacgacgca	840
tgggtgaagt tcaacagggt tcgtagagag atgacctga ctgtgctcga tcttatcggt	900
ctctttccat tctacgacat tcgtctttac tccaaaggcg ttaagacaga gctgaccaga	960
gacatcttca cggatcccat cttctcactt aacaccctgc aggaatacgg tccaactttt	1020
ctctccattg agaacagcat cagggaagcct cacctcttcg actatctgca aggcattgag	1080
tttcacacca ggttgcaacc tggttacttc ggtaaggatt ccttcaacta ctggagcgga	1140
aactacgttg aaaccagacc atccatcgga tctagcaaga ccatcacttc tccattctac	1200
ggtgacaaga gcaactgagc agtgcagaag ttgagcttcg atgggcagaa ggtgtataga	1260
accatcgcca ataccgatgt tgcagcttgg cctaattggca aggtctacct tggagtact	1320
aaagtggact tctcccaata cgacgatcag aagaacgaga catctactca aacctacgat	1380
agtaagagga acaatggcca tgtttccgca caagactcca ttgaccaact tccacctgaa	1440
accactgatg aaccattgga gaaggcttac agtcaccaac ttaactacgc cgaatgcttt	1500
ctcatgcaag acaggcgtgg caccattccg ttctttacat ggactcacag gtctgtcgac	1560
ttctttaaca ctatcgacgc tgagaagatt acccaacttc ccgtgggtcaa ggcttatgcc	1620
ttgtccagcg gagcttccat cattgaaggt ccaggcttca ccggtggcaa cttgctcttc	1680
cttaaggagt ccagcaactc catcgccaag ttcaaagtga cacttaactc agcagccttg	1740
ctccaacggt acagggttcg tatcagatag gcaagcacta ccaatcttcg cctctttgtc	1800
cagaacagca acaatgattt ccttgtcatc tacatcaaca agactatgaa caaagacgat	1860
gacctcacct acaacacatt cgatcttgcc actaccaata gtaacatggg attctctggt	1920
gacaagaacg agctgatcat aggtgtgtag agctttgtct ctaatgagaa gatttacata	1980
gacaagatcg agttcattcc agttcaactc taatagatcc cccgggctgc aggaattcga	2040
tatcaagctt	2050

<210> SEQ ID NO 102

<211> LENGTH: 2280

<212> TYPE: DNA

<213> ORGANISM: Bacillus thuringiensis

<400> SEQUENCE: 102

ttaaaattaa ttttgtatag ttttcattgt aataatatga ttttaaaac gaaaaagtgc	60
atatacaact tatcaggagg ggggggatgc acaaagaaga aaagaataag aagtgaatgt	120
ttataatggt caatagtttt atgggaaggc attttatcag gtgaaaagtt atgtattatg	180
ataagaatgg gaggaagaaa aatgaatcca aacaatcgaa gtgaacatga tacgataaag	240
gttacaccta acagtgaatt gcaaactaac cataatcaat atccttttagc tgacaatcca	300
aattcaacac tagaagaatt aaattataaa gaatttttaa gaatgactga agacagttct	360
acggaagtgc tagacaactc tacagtaaaa gatgcagttg ggacaggaat ttctgttgta	420
gggcagatgt taggtgttgt aggagttcca tttgctgggg cactcacttc attttatcaa	480
tcattttcta acactatatg gccaaagtgt gctgacctat ggaaggcttt tatggcacia	540

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gttgaagtac tgatagataa gaaaatagag gagtatgcta aaagtaaagc tcttgcaagag 600
ttacagggtc ttcaaaataa tttcgaagat tatgttaatg cgttaaattc ctggaagaaa 660
acacctttaa gtttgcgaag taaaagaagc caagatcgaa taagggaact tttttctcaa 720
gcagaaagtc attttcgtaa ttccatgccg tcatttgtag tttccaaatt cgaagtgtctg 780
tttctaccaa catatgcaca agctgcgaat acacatttat tgctattaaa agatgctcaa 840
gtttttggag aagaatgggg atattcttca gaagatgttg ctgaatttta tcatagacaa 900
ttaaacttta cacaacaata cactgacat tgtgttaatt ggtataatgt tggattaaat 960
ggtttaagag gttcaactta tgatgcattg gtcaaattta accgttttcg cagagaaatg 1020
actttaactg tattagatct aattgtactt ttccattttt atgatatctg gttatactca 1080
aaaggggtta aaacagaact aacaagagac atttttacgg atccaatttt ttcacttaat 1140
actcttcagg agtatggacc aacttttttg agtatagaaa actctattcg aaaacctcat 1200
ttatttgatt atttacaggg gattgaattt catacgcgtc ttcaacctgg ttactttggg 1260
aaagattctt tcaattattg gtctggtaat tatgtagaaa ctgacacctg tataggatct 1320
agtaagacaa ttacttcccc attttatgga gataaatcta ctgaacctgt acaaaagcta 1380
agctttgatg gacaaaaagt ttatcgaact atagctaata cagacgtagc ggcttgccg 1440
aatggtaag tatlattagg tgttacgaaa gttgatttta gtcaatatga tgatcaaaaa 1500
aatgaaacta gtacacaaac atatgattca aaaagaaaca atggccatgt aagtgcacag 1560
gattctattg accaattacc gccagaaaca acagatgaac cacttgaaaa agcatatagt 1620
catcagctta attacggga atgtttctta atgcaggacc gtcgtggaac aattccattt 1680
tttacttgga cacatagaag tgtagacttt tttaatacaa ttgatgctga aaagattact 1740
caacttccag tagtgaaagc atatgccttg tcttcagggtg cttccattat tgaagggtcca 1800
ggattcacag gaggaatttt actattccta aaagaatcta gtaattcaat tgctaaattt 1860
aaagttacat taaattcagc agccttggtt caacgataac gtgtaagaat acgctatgct 1920
tctaccacta acttacgact tttgtgcaa aattcaaaca atgattttct tgcacatctac 1980
attaataaaa ctatgaataa agatgatgat ttaacatata aaacatttga tctcgcaact 2040
actaattcta atatgggggt ctcgggtgat aagaatgaac ttataatagg agcagaatct 2100
ttcgtttcta atgaaaaaat ctatatagat aagatagaat ttatcccagt acaattgtaa 2160
ggagatttta aaatgttggg tgatgggtcaa aatgaaagaa taggaagggtg aattttgatg 2220
gttaggaaag attcttttaa caaaagcaac atggaaaagt atacagtaca aatattaacc 2280

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<210> SEQ ID NO 103
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

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<400> SEQUENCE: 103

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taggcctcca tccatggcaa accctaacaa tc

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32

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<210> SEQ ID NO 104
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Oligonucleotide

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<400> SEQUENCE: 104

tcccacatcttc ctactttacga ccctgcagaa atacgggtcca ac	42
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<210> SEQ ID NO 105

<211> LENGTH: 28

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Oligonucleotide

<400> SEQUENCE: 105

gacctcacct accaaacatt cgatcttg	28
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<210> SEQ ID NO 106

<211> LENGTH: 25

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Oligonucleotide

<400> SEQUENCE: 106

cgagttctac cgtaggcagc tcaag	25
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<210> SEQ ID NO 107

<211> LENGTH: 1959

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 107

atgaatccaa acaatcgaag tgaacatgat acgataaagg ttacacctaa cagtgaattg	60
caaaactaacc ataatacaata tccttttagct gacaatccaa attcaacact agaagaatta	120
aattataaag aattttttaag aatgactgaa gacagttcta cggaagtgtc agacaactct	180
acagtaaaaag atgcagttgg gacaggaatt totgtttagt ggcagatttt aggtgttgta	240
ggagttccat ttgctggggc actcacttca ttttatcaat catttcttaa cactatatgg	300
ccaagtgatg ctgacccatg gaaggctttt atggcacaag ttgaagtact gatagataag	360
aaaatagagg agtatgctaa aagtaaaagt cttgcagagt tacagggtct tcaaaataat	420
ttcgaagatt atgttaatgc gttaaattcc tggaagaaaa cacctttaag tttgcgaagt	480
aaaagaagcc aaggtcgaat aagggaaatt ttttctcaag cagaaagtca ttttcgtaat	540
tccatgccgt catittgcagt ttccaaattc gaagtgtgtt ttctaccaac atatgcacaa	600
gctgcaaata cacatttatt gctattaaaa gatgctcaag tttttggaga agaattggga	660
tattcttcag aagatgttgc tgaattctat cgtagacaat taaaacttac acaacaatac	720
actgaccatt gtgttaattg gtataatgtt ggattaaatg gtttaagagg ttcaacttat	780
gatgcatggg tcaaatttaa cgtttttcgc agagaaatga ctttaactgt attagatcta	840
attgtacttt tcccatttta tgatattcgg ttatactcaa aaggggttaa aacagaacta	900
acaagagaca tttttacgga tccaattttt ttacttacta cgcttcagaa gtacggacca	960
acttttttga gtatagaaaa ctctattcga aaacotcatt tatttgatta tttacagggg	1020
attgaatttc atacgcgtct tcaacctggt tactttggga aagattcttt caattattgg	1080
tctggtaatt atgtagaac tagacctagt ataggatcta gtaagacaat tacttcccca	1140
ttttatggag ataaatctac tgaacctgta caaaagctaa gctttgatgg acaaaaagtt	1200
tatcgaacta tagctaatac agacgtagcg gcttggccga atggtaaggt atatttaggt	1260

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gttacgaaag ttgatttttag tcaatatgat gatcaaaaaa atgaaactag tacacaaaca 1320
tatgattcaa aaagaacaaa tggccatgta agtgcacagg attctattga ccaattaccg 1380
ccagaaacaa cagatgaacc acttgaaaaa gcatatagtc atcagcttaa ttacgcggaa 1440
tgtttcttaa tgcaggaccg tcgtggaaca attccatttt ttacttggac acatagaagt 1500
gtagactttt ttaataacaat tgatgctgaa aagattactc aacttccagt agtgaagca 1560
tatgccttgt cttcaggtgc ttccattatt gaaggtccag gattcacagg aggaaattta 1620
ctattcctaa aagaatctag taattcaatt gctaaattta aagttacatt aaattcagca 1680
gccttggtac aacgatatcg tgtaagaata cgctatgctt ctaccactaa cttacgactt 1740
tttgtgcaaa attcaacaaa tgattttctt gtcactaca ttaataaaac tatgaataaa 1800
gatgatgatt taacatatca aacatttgat ctgcgaacta ctaattctaa tatgggggtc 1860
tcgggtgata agaatgaact tataatagga gcagaatctt tcgtttctaa tgaaaaaatc 1920
tatatagata agatagaatt tatcccagta caattgtaa 1959

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<210> SEQ ID NO 108

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 108

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Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
1           5           10           15

Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
20          25          30

Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
35          40          45

Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
50          55          60

Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
65          70          75          80

Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
85          90          95

Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
100         105         110

Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
115         120         125

Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
130         135         140

Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
145         150         155         160

Lys Arg Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser
165         170         175

His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val
180         185         190

Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
195         200         205

Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
210         215         220

Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln Gln Tyr
225         230         235         240

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Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg	245	250	255
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu	260	265	270
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp	275	280	285
Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile	290	295	300
Phe	Thr	Asp	Pro	Ile	Phe	Leu	Leu	Thr	Thr	Leu	Gln	Lys	Tyr	Gly	Pro	305	310	315
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	325	330	335
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe	340	345	350
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg	355	360	365
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp	370	375	380
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val	385	390	395
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys	405	410	415
Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln	420	425	430
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly	435	440	445
His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	450	455	460
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu	465	470	475
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp	485	490	495
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile	500	505	510
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser	515	520	525
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys	530	535	540
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala	545	550	555
Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr	565	570	575
Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile	580	585	590
Tyr	Ile	Asn	Lys	Thr	Met	Asn	Lys	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr	595	600	605
Phe	Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys	610	615	620
Asn	Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile	625	630	635
Tyr	Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu					645	650	

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<210> SEQ ID NO 109
<211> LENGTH: 649
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 109

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Ala Thr Glu
1          5          10          15
Asn Asn Glu Val Ser Asn Asn His Ala Gln Tyr Pro Leu Ala Asp Thr
20          25          30
Pro Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Thr Thr
35          40          45
Asp Asn Asn Val Glu Ala Leu Asp Ser Ser Thr Thr Lys Asp Ala Ile
50          55          60
Gln Lys Gly Ile Ser Ile Ile Gly Asp Leu Leu Gly Val Val Gly Phe
65          70          75          80
Pro Tyr Gly Gly Ala Leu Val Ser Phe Tyr Thr Asn Leu Leu Asn Thr
85          90          95
Ile Trp Pro Gly Glu Asp Pro Leu Lys Ala Phe Met Gln Gln Val Glu
100         105         110
Ala Leu Ile Asp Gln Lys Ile Ala Asp Tyr Ala Lys Asp Lys Ala Thr
115         120         125
Ala Glu Leu Gln Gly Leu Lys Asn Val Phe Lys Asp Tyr Val Ser Ala
130         135         140
Leu Asp Ser Trp Asp Lys Thr Pro Leu Thr Leu Arg Asp Gly Arg Ser
145         150         155         160
Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His Phe Arg
165         170         175
Arg Ser Met Pro Ser Phe Ala Val Ser Gly Tyr Glu Val Leu Phe Leu
180         185         190
Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu Leu Lys Asp
195         200         205
Ala Gln Ile Tyr Gly Thr Asp Trp Gly Tyr Ser Thr Asp Asp Leu Asn
210         215         220
Glu Phe His Thr Lys Gln Lys Asp Leu Thr Ile Glu Tyr Thr Asn His
225         230         235         240
Cys Ala Lys Trp Tyr Lys Ala Gly Leu Asp Lys Leu Arg Gly Ser Thr
245         250         255
Tyr Glu Glu Trp Val Lys Phe Asn Arg Tyr Arg Arg Glu Met Thr Leu
260         265         270
Thr Val Leu Asp Leu Ile Thr Leu Phe Pro Leu Tyr Asp Val Arg Thr
275         280         285
Tyr Thr Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Val Leu Thr Asp
290         295         300
Pro Ile Val Ala Val Asn Asn Met Asn Gly Tyr Gly Thr Thr Phe Ser
305         310         315         320
Asn Ile Glu Asn Tyr Ile Arg Lys Pro His Leu Phe Asp Tyr Leu His
325         330         335
Ala Ile Gln Phe His Ser Arg Leu Gln Pro Gly Tyr Phe Gly Thr Asp
340         345         350
Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Ser Thr Arg Ser Ser Ile
355         360         365

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Gly Ser Asp Glu Ile Ile Arg Ser Pro Phe Tyr Gly Asn Lys Ser Thr
 370 375 380
 Leu Asp Val Gln Asn Leu Glu Phe Asn Gly Glu Lys Val Phe Arg Ala
 385 390 395 400
 Val Ala Asn Gly Asn Leu Ala Val Trp Pro Val Gly Thr Gly Gly Thr
 405 410 415
 Lys Ile His Ser Gly Val Thr Lys Val Gln Phe Ser Gln Tyr Asn Asp
 420 425 430
 Arg Lys Asp Glu Val Arg Thr Gln Thr Tyr Asp Ser Lys Arg Asn Val
 435 440 445
 Gly Gly Ile Val Phe Asp Ser Ile Asp Gln Leu Pro Pro Ile Thr Thr
 450 455 460
 Asp Glu Ser Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Val Arg
 465 470 475 480
 Cys Phe Leu Leu Gln Gly Gly Arg Gly Ile Ile Pro Val Phe Thr Trp
 485 490 495
 Thr His Lys Ser Val Asp Phe Tyr Asn Thr Leu Asp Ser Glu Lys Ile
 500 505 510
 Thr Gln Ile Pro Phe Val Lys Ala Phe Ile Leu Val Asn Ser Thr Ser
 515 520 525
 Val Val Ala Gly Pro Gly Phe Thr Gly Gly Asp Ile Ile Lys Cys Thr
 530 535 540
 Asn Gly Ser Gly Leu Thr Leu Tyr Val Thr Pro Ala Pro Asp Leu Thr
 545 550 555 560
 Tyr Ser Lys Thr Tyr Lys Ile Arg Ile Arg Tyr Ala Ser Thr Ser Gln
 565 570 575
 Val Arg Phe Gly Ile Asp Leu Gly Ser Tyr Thr His Ser Ile Ser Tyr
 580 585 590
 Phe Asp Lys Thr Met Asp Lys Gly Asn Thr Leu Thr Tyr Asn Ser Phe
 595 600 605
 Asn Leu Ser Ser Val Ser Arg Pro Ile Glu Ile Ser Gly Gly Asn Lys
 610 615 620
 Ile Gly Val Ser Val Gly Gly Ile Gly Ser Gly Asp Glu Val Tyr Ile
 625 630 635 640
 Asp Lys Ile Glu Phe Ile Pro Met Asp
 645

<210> SEQ ID NO 110

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 110

Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro
 1 5 10 15
 Asn Ser Glu Leu Pro Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
 20 25 30
 Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
 35 40 45
 Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
 50 55 60
 Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
 65 70 75 80

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Gly	Val	Pro	Phe	Ala	Gly	Ala	Leu	Thr	Ser	Phe	Tyr	Gln	Ser	Phe	Leu
			85						90					95	
Asp	Thr	Ile	Trp	Pro	Ser	Asp	Ala	Asp	Pro	Trp	Lys	Ala	Phe	Met	Ala
			100					105					110		
Gln	Val	Glu	Val	Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser
		115					120					125			
Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr
	130					135					140				
Val	Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Thr	Pro	Leu	Ser	Leu	Arg	Ser
145				150						155				160	
Lys	Arg	Ser	Gln	Asp	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser
			165					170						175	
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val
		180						185					190		
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu
	195						200					205			
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu
	210					215					220				
Asp	Val	Ala	Glu	Phe	Tyr	His	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr
225				230						235					240
Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg
			245					250						255	
Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu
		260					265					270			
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp
	275					280						285			
Val	Arg	Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile
	290				295					300					
Phe	Thr	Asp	Pro	Ile	Phe	Ser	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro
305				310						315					320
Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp
		325						330						335	
Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Ser
		340					345					350			
Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg
	355					360						365			
Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp
	370				375						380				
Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val
385				390						395					400
Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys
		405						410						415	
Ile	Tyr	Phe	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln
		420					425					430			
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly
	435					440						445			
His	Val	Gly	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr
	450				455						460				
Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu
465				470						475					480
Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp
		485						490						495	
Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile

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500					505					510					
Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser
		515					520						525		
Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys
		530				535					540				
Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala
		545				550					555				560
Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr
			565						570					575	
Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Ile	Val	Ile
			580					585					590		
Tyr	Ile	Asn	Lys	Thr	Met	Asn	Ile	Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr
		595					600					605			
Phe	Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp	Thr
		610					615					620			
Asn	Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile
		625				630					635				640
Tyr	Ile	Asp	Lys	Ile	Glu	Phe	Ile	Pro	Val	Gln	Leu				
			645					650							

<210> SEQ ID NO 111

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 111

Met	Asn	Pro	Asn	Asn	Arg	Ser	Glu	His	Asp	Thr	Ile	Lys	Val	Thr	Pro
1				5					10					15	
Asn	Ser	Glu	Leu	Gln	Thr	Asn	His	Asn	Gln	Tyr	Pro	Leu	Ala	Asp	Asn
		20					25						30		
Pro	Asn	Ser	Thr	Leu	Glu	Glu	Leu	Asn	Tyr	Lys	Glu	Phe	Leu	Arg	Met
		35					40					45			
Thr	Glu	Asp	Ser	Ser	Thr	Glu	Val	Leu	Asp	Asn	Ser	Thr	Val	Lys	Asp
		50				55					60				
Ala	Val	Gly	Thr	Gly	Ile	Ser	Val	Val	Gly	Gln	Ile	Leu	Gly	Val	Val
		65			70					75				80	
Gly	Val	Pro	Phe	Ala	Gly	Ala	Leu	Thr	Ser	Phe	Tyr	Gln	Ser	Phe	Leu
			85					90						95	
Asn	Thr	Ile	Trp	Pro	Ser	Asp	Ala	Asp	Pro	Trp	Lys	Ala	Phe	Met	Ala
		100					105						110		
Gln	Val	Glu	Val	Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser
		115					120					125			
Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr
		130				135						140			
Val	Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Thr	Pro	Leu	Ser	Leu	Arg	Ser
		145			150					155				160	
Lys	Arg	Ser	Gln	Asp	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser
			165					170						175	
His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val
		180						185					190		
Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu
		195					200					205			
Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu

210					215					220					
Asp 225	Val	Ala	Glu	Phe	Tyr 230	His	Arg	Gln	Leu	Lys 235	Leu	Thr	Gln	Gln	Tyr 240
Thr	Asp	His	Cys	Val 245	Asn	Trp	Tyr	Asn	Val 250	Gly	Leu	Asn	Gly	Leu	Arg 255
Gly	Ser	Thr	Tyr 260	Asp	Ala	Trp	Val	Lys 265	Phe	Asn	Arg	Phe	Arg	Arg	Glu
Met	Thr	Leu	Thr	Val	Leu	Asp	Leu 280	Ile	Val	Leu	Phe	Pro 285	Phe	Tyr	Asp
Ile	Arg	Leu	Tyr	Ser	Lys 295	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile
Phe 305	Thr	Asp	Pro	Ile	Phe 310	Ser	Leu	Asn	Thr	Leu	Gln	Glu	Tyr	Gly	Pro 320
Thr	Phe	Leu	Ser	Ile 325	Glu	Asn	Ser	Ile	Arg 330	Lys	Pro	His	Leu	Phe	Asp 335
Tyr	Leu	Gln	Gly 340	Ile	Glu	Phe	His	Thr 345	Arg	Leu	Gln	Pro	Gly	Tyr	Phe 350
Gly	Lys	Asp 355	Ser	Phe	Asn	Tyr	Trp 360	Ser	Gly	Asn	Tyr	Val 365	Glu	Thr	Arg
Pro	Ser	Ile	Gly	Ser	Ser	Lys 375	Thr	Ile	Thr	Ser	Pro 380	Phe	Tyr	Gly	Asp
Lys 385	Ser	Thr	Glu	Pro	Val 390	Gln	Lys	Leu	Ser	Phe	Asp 395	Gly	Gln	Lys	Val 400
Tyr	Arg	Thr	Ile	Ala 405	Asn	Thr	Asp	Val	Ala 410	Ala	Trp	Pro	Asn	Gly	Lys 415
Val	Tyr	Leu	Gly 420	Val	Thr	Lys	Val	Asp 425	Phe	Ser	Gln	Tyr	Asp 430	Asp	Gln
Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr 440	Tyr	Asp	Ser	Lys	Arg 445	Asn	Asn	Gly
His	Val	Ser	Ala	Gln	Asp	Ser	Ile 455	Asp	Gln	Leu	Pro 460	Pro	Glu	Thr	Thr
Asp 465	Glu	Pro	Leu	Glu	Lys 470	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu 480
Cys	Phe	Leu	Met	Gln 485	Asp	Arg	Arg	Gly	Thr 490	Ile	Pro	Phe	Phe	Thr	Trp 495
Thr	His	Arg	Ser	Val 500	Asp	Phe	Phe	Asn	Thr 505	Ile	Asp	Ala	Glu	Lys	Ile
Thr	Gln	Leu	Pro	Val 515	Val	Lys	Ala 520	Tyr	Ala	Leu	Ser	Ser 525	Gly	Ala	Ser
Ile	Ile	Glu	Gly	Pro	Gly 535	Phe	Thr	Gly	Gly	Asn	Leu 540	Leu	Phe	Leu	Lys
Glu 545	Ser	Ser	Asn	Ser	Ile 550	Ala	Lys	Phe	Lys	Val 555	Thr	Leu	Asn	Ser	Ala 560
Ala	Leu	Leu	Gln	Arg 565	Tyr	Arg	Val	Arg	Ile 570	Arg	Tyr	Ala	Ser	Thr	Thr 575
Asn	Leu	Arg	Leu	Phe	Val 580	Gln	Asn	Ser 585	Asn	Asn	Asp	Phe	Leu	Val	Ile
Tyr	Ile	Asn	Lys	Thr	Met	Asn	Lys 600	Asp	Asp	Asp	Leu	Thr 605	Tyr	Gln	Thr
Phe	Asp	Leu	Ala	Thr	Thr	Asn 615	Ser	Asn	Met	Gly	Phe 620	Ser	Gly	Asp	Lys
Asn 625	Glu	Leu	Ile	Ile	Gly 630	Ala	Glu	Ser	Phe	Val 635	Ser	Asn	Glu	Lys	Ile 640

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Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> SEQ ID NO 112
 <211> LENGTH: 659
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 112

Met Ile Arg Met Gly Gly Arg Lys Met Asn Pro Asn Asn Arg Ser Glu
 1 5 10 15

Tyr Asp Thr Ile Lys Val Thr Pro Asn Ser Glu Leu Pro Thr Asn His
 20 25 30

Asn Gln Tyr Pro Leu Ala Asp Asn Pro Asn Ser Thr Leu Glu Glu Leu
 35 40 45

Asn Tyr Lys Glu Phe Leu Arg Met Thr Ala Asp Asn Ser Thr Glu Val
 50 55 60

Leu Asp Ser Ser Thr Val Lys Asp Ala Val Gly Thr Gly Ile Ser Val
 65 70 75 80

Val Gly Gln Ile Leu Gly Val Val Gly Val Pro Phe Ala Gly Ala Leu
 85 90 95

Thr Ser Phe Tyr Gln Ser Phe Leu Asn Ala Ile Trp Pro Ser Asp Ala
 100 105 110

Asp Pro Trp Lys Ala Phe Met Ala Gln Val Glu Val Leu Ile Asp Lys
 115 120 125

Lys Ile Glu Glu Tyr Ala Lys Ser Lys Ala Leu Ala Glu Leu Gln Gly
 130 135 140

Leu Gln Asn Asn Phe Glu Asp Tyr Val Asn Ala Leu Asp Ser Trp Lys
 145 150 155 160

Lys Ala Pro Val Asn Leu Arg Ser Arg Arg Ser Gln Asp Arg Ile Arg
 165 170 175

Glu Leu Phe Ser Gln Ala Glu Ser His Phe Arg Asn Ser Met Pro Ser
 180 185 190

Phe Ala Val Ser Lys Phe Glu Val Leu Phe Leu Pro Thr Tyr Ala Gln
 195 200 205

Ala Ala Asn Thr His Leu Leu Leu Leu Lys Asp Ala Gln Val Phe Gly
 210 215 220

Glu Glu Trp Gly Tyr Ser Ser Glu Asp Ile Ala Glu Phe Tyr Gln Arg
 225 230 235 240

Gln Leu Lys Leu Thr Gln Gln Tyr Thr Asp His Cys Val Asn Trp Tyr
 245 250 255

Asn Val Gly Leu Asn Ser Leu Arg Gly Ser Thr Tyr Asp Ala Trp Val
 260 265 270

Lys Phe Asn Arg Phe Arg Arg Glu Met Thr Leu Thr Val Leu Asp Leu
 275 280 285

Ile Val Leu Phe Pro Phe Tyr Asp Val Arg Leu Tyr Ser Lys Gly Val
 290 295 300

Lys Thr Glu Leu Thr Arg Asp Ile Phe Thr Asp Pro Ile Phe Thr Leu
 305 310 315 320

Asn Ala Leu Gln Glu Tyr Gly Pro Thr Phe Ser Ser Ile Glu Asn Ser
 325 330 335

Ile Arg Lys Pro His Leu Phe Asp Tyr Leu Arg Gly Ile Glu Phe His
 340 345 350

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Thr Arg Leu Arg Pro Gly Tyr Ser Gly Lys Asp Ser Phe Asn Tyr Trp
 355 360 365
 Ser Gly Asn Tyr Val Glu Thr Arg Pro Ser Ile Gly Ser Asn Asp Thr
 370 375 380
 Ile Thr Ser Pro Phe Tyr Gly Asp Lys Ser Ile Glu Pro Ile Gln Lys
 385 390 395 400
 Leu Ser Phe Asp Gly Gln Lys Val Tyr Arg Thr Ile Ala Asn Thr Asp
 405 410 415
 Ile Ala Ala Phe Pro Asp Gly Lys Ile Tyr Phe Gly Val Thr Lys Val
 420 425 430
 Asp Phe Ser Gln Tyr Asp Asp Gln Lys Asn Glu Thr Ser Thr Gln Thr
 435 440 445
 Tyr Asp Ser Lys Arg Tyr Asn Gly Tyr Leu Gly Ala Gln Asp Ser Ile
 450 455 460
 Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro Leu Glu Lys Ala Tyr
 465 470 475 480
 Ser His Gln Leu Asn Tyr Ala Glu Cys Phe Leu Met Gln Asp Arg Arg
 485 490 495
 Gly Thr Ile Pro Phe Phe Thr Trp Thr His Arg Ser Val Asp Phe Phe
 500 505 510
 Asn Thr Ile Asp Ala Glu Lys Ile Thr Gln Leu Pro Val Val Lys Ala
 515 520 525
 Tyr Ala Leu Ser Ser Gly Ala Ser Ile Ile Glu Gly Pro Gly Phe Thr
 530 535 540
 Gly Gly Asn Leu Leu Phe Leu Lys Glu Ser Ser Asn Ser Ile Ala Lys
 545 550 555 560
 Phe Lys Val Thr Leu Asn Ser Ala Ala Leu Leu Gln Arg Tyr Arg Val
 565 570 575
 Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu Arg Leu Phe Val Gln Asn
 580 585 590
 Ser Asn Asn Asp Phe Leu Val Ile Tyr Ile Asn Lys Thr Met Asn Ile
 595 600 605
 Asp Gly Asp Leu Thr Tyr Gln Thr Phe Asp Phe Ala Thr Ser Asn Ser
 610 615 620
 Asn Met Gly Phe Ser Gly Asp Thr Asn Asp Phe Ile Ile Gly Ala Glu
 625 630 635 640
 Ser Phe Val Ser Asn Glu Lys Ile Tyr Ile Asp Lys Ile Glu Phe Ile
 645 650 655
 Pro Val Gln

<210> SEQ ID NO 113

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant delta endotoxin

<400> SEQUENCE: 113

Met Ile Arg Lys Gly Gly Arg Lys Met Asn Pro Asn Asn Arg Ser Glu
 1 5 10 15
 His Asp Thr Ile Lys Thr Thr Glu Asn Asn Glu Val Pro Thr Asn His
 20 25 30
 Val Gln Tyr Pro Leu Ala Glu Thr Pro Asn Pro Thr Leu Glu Asp Leu
 35 40 45

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Asn	Tyr	Lys	Glu	Phe	Leu	Arg	Met	Thr	Ala	Asp	Asn	Asn	Thr	Glu	Ala
50						55				60					
Leu	Asp	Ser	Ser	Thr	Thr	Lys	Asp	Val	Ile	Gln	Lys	Gly	Ile	Ser	Val
65					70					75					80
Val	Gly	Asp	Leu	Leu	Gly	Val	Val	Gly	Phe	Pro	Phe	Gly	Gly	Ala	Leu
			85						90					95	
Val	Ser	Phe	Tyr	Thr	Asn	Phe	Leu	Asn	Thr	Ile	Trp	Pro	Ser	Glu	Asp
			100					105					110		
Pro	Trp	Lys	Ala	Phe	Met	Glu	Gln	Val	Glu	Ala	Leu	Met	Asp	Gln	Lys
		115					120					125			
Ile	Ala	Asp	Tyr	Ala	Lys	Asn	Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu
	130					135					140				
Gln	Asn	Asn	Val	Glu	Asp	Tyr	Val	Ser	Ala	Leu	Ser	Ser	Trp	Gln	Lys
145					150					155					160
Asn	Pro	Val	Ser	Ser	Arg	Asn	Pro	His	Ser	Gln	Gly	Arg	Ile	Arg	Glu
				165					170					175	
Leu	Phe	Ser	Gln	Ala	Glu	Ser	His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe
			180					185					190		
Ala	Ile	Ser	Gly	Tyr	Glu	Val	Leu	Phe	Leu	Thr	Thr	Tyr	Ala	Gln	Ala
		195					200					205			
Ala	Asn	Thr	His	Leu	Phe	Leu	Leu	Lys	Asp	Ala	Gln	Ile	Tyr	Gly	Glu
	210					215				220					
Glu	Trp	Gly	Tyr	Glu	Lys	Glu	Asp	Ile	Ala	Glu	Phe	Tyr	Lys	Arg	Gln
225					230					235					240
Leu	Lys	Leu	Thr	Gln	Glu	Tyr	Thr	Asp	His	Cys	Val	Lys	Trp	Tyr	Asn
			245						250					255	
Val	Gly	Leu	Asp	Lys	Leu	Arg	Gly	Ser	Ser	Tyr	Glu	Ser	Trp	Val	Asn
		260						265					270		
Phe	Asn	Arg	Tyr	Arg	Arg	Glu	Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile
		275					280					285			
Ala	Leu	Phe	Pro	Leu	Tyr	Asp	Val	Arg	Leu	Tyr	Pro	Lys	Glu	Val	Lys
	290					295					300				
Thr	Glu	Leu	Thr	Arg	Asp	Val	Leu	Thr	Asp	Pro	Ile	Val	Gly	Val	Asn
305					310					315					320
Asn	Leu	Arg	Gly	Tyr	Gly	Thr	Thr	Phe	Ser	Asn	Ile	Glu	Asn	Tyr	Ile
			325						330					335	
Arg	Lys	Pro	His	Leu	Phe	Asp	Tyr	Leu	His	Arg	Ile	Gln	Phe	His	Thr
			340					345					350		
Arg	Phe	Gln	Pro	Gly	Tyr	Tyr	Gly	Asn	Asp	Ser	Phe	Asn	Tyr	Trp	Ser
		355					360					365			
Gly	Asn	Tyr	Val	Ser	Thr	Arg	Pro	Ser	Ile	Gly	Ser	Asn	Asp	Ile	Ile
	370					375					380				
Thr	Ser	Pro	Phe	Tyr	Gly	Asn	Lys	Ser	Ser	Glu	Pro	Val	Gln	Asn	Leu
385					390					395					400
Glu	Phe	Asn	Gly	Glu	Lys	Val	Tyr	Arg	Ala	Val	Ala	Asn	Thr	Asn	Leu
			405						410					415	
Ala	Val	Trp	Pro	Ser	Ala	Val	Tyr	Ser	Gly	Val	Thr	Lys	Val	Glu	Phe
			420					425					430		
Ser	Gln	Tyr	Asn	Asp	Gln	Thr	Asp	Glu	Ala	Ser	Thr	Gln	Thr	Tyr	Asp
		435					440					445			
Ser	Lys	Arg	Asn	Val	Gly	Ala	Val	Ser	Trp	Asp	Ser	Ile	Asp	Gln	Leu
	450				455					460					
Pro	Pro	Glu	Thr	Thr	Asp	Glu	Pro	Leu	Glu	Lys	Gly	Tyr	Ser	His	Gln

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465	470	475	480
Leu Asn Tyr Val Met Cys Phe Leu Met Gln Gly Ser Arg Gly Thr Ile	485	490	495
Pro Val Leu Thr Trp Thr His Lys Ser Val Asp Phe Phe Asn Met Ile	500	505	510
Asp Ser Lys Lys Ile Thr Gln Leu Pro Leu Val Lys Ala Tyr Lys Leu	515	520	525
Gln Ser Gly Ala Ser Val Val Ala Gly Pro Arg Phe Thr Gly Gly Asp	530	535	540
Ile Ile Gln Cys Thr Glu Asn Gly Ser Ala Ala Thr Ile Tyr Val Thr	545	550	555
Pro Asp Val Ser Tyr Ser Gln Lys Tyr Arg Ala Arg Ile His Tyr Ala	565	570	575
Ser Thr Ser Gln Ile Thr Phe Thr Leu Ser Leu Asp Gly Ala Pro Phe	580	585	590
Asn Gln Tyr Tyr Phe Asp Lys Thr Ile Asn Lys Gly Asp Thr Leu Thr	595	600	605
Tyr Asn Ser Phe Asn Leu Ala Ser Phe Ser Thr Pro Phe Glu Leu Ser	610	615	620
Gly Asn Asn Leu Gln Ile Gly Val Thr Gly Leu Ser Ala Gly Asp Lys	625	630	635
Val Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Asn	645	650	

What is claimed is:

1. A transgenic plant having incorporated into its genome a *modified cry3B** gene that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, and SEQ ID NO:108.

2. A transgenic plant having incorporated into its genome a *modified cry3B** gene comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, and SEQ ID NO:107.

3. A progeny or seed from the transgenic plant of claim 1 or claim 2 comprising [a] the modified *cry3B** gene.

4. A seed from the progeny of claim 3 comprising [a] the modified *cry3B** gene.

5. A plant from the progeny or seed of claim 3 [or claim 4] comprising [a] the modified *cry3B** gene.

6. A host cell having an NRRL accession number selected from the group consisting of NRRL B-21744, NRRL B-21745, NRRL B-21746, NRRL B-21747, NRRL B-21748, NRRL B-21749, NRRL B-21750, NRRL B-21751, NRRL B-21752, NRRL B-21753, NRRL B-21754, NRRL B-21755, NRRL B-21756, NRRL B-21757, NRRL B-21758, NRRL B-21759, NRRL B-21760, NRRL B-21761, NRRL B-21762, NRRL B-21763, NRRL B-21764, NRRL B-21765, NRRL B-21766, NRRL B-21767, NRRL B-21768, NRRL B-21769, NRRL B-21770, NRRL B-21771, NRRL B-21772, NRRL B-21773, NRRL B-21774, NRRL B-21775, NRRL B-21776, NRRL B-21777, NRRL B-21778, and NRRL B-21779.

7. A host cell selected from the group consisting of an EG11221, EG11222, EG11223, EG11224, EG11225, EG11226, EG11227, EG11228, EG11229, EG11230, EG11231, EG11232, EG11233, EG11234, EG11235, EG11236, EG11237, EG11238, EG11239, EG11241, EG11242, EG11032, EG11035, EG11036, EG11046, EG11048, EG11051, EG11057, EG11058, EG11081, EG11082, EG11083, EG11084, EG11095, and an EG11098 cell.

8. A transformed bacterial or plant host cell comprising a modified *cry3B** gene that encodes a modified *Cry3B** protein having enhanced insecticidal activity against a Coleopteran insect pest relative to an unmodified [*Cry3B**] *Cry3B* protein, wherein said modified *Cry3B** protein comprises the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46,

SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, or SEQ ID NO:108.

9. The host cell of claim 8, wherein said host cell is an E. coli, B. thuringiensis, A. tumefaciens, B. subtilis, B. megaterium, B. cereus, Salmonella spp., or Pseudomonas spp. cell.

10. The host cell of claim 9, wherein said B. thuringiensis cell has an NRRL accession number selected from the group consisting of NRRL B-21744, NRRL B-21745, NRRL B-21746, NRRL B-21747, NRRL B-21748, NRRL B-21749, NRRL B-21750, NRRL B-21751, NRRL B-21752, NRRL B-21753, NRRL B-21754, NRRL B-21755, NRRL B-21756, NRRL B-21757, NRRL B-21758, NRRL B-21759, NRRL B-21760, NRRL B-21761, NRRL B-21762, NRRL B-21763, NRRL B-21764, NRRL B-21765, NRRL B-21766, NRRL B-21767, NRRL B-21768, NRRL B-21769, NRRL B-21770, NRRL B-21771, NRRL B-21772, NRRL B-21773, NRRL B-21774, NRRL B-21775, NRRL B-21776, NRRL B-21777, NRRL B-21778, and NRRL B-21779.

11. The host cell of claim 8, wherein said host cell is a plant cell selected from the group consisting of a grain, tree, legume, vegetable, fruit, berry, nut, citrus, grass, cactus, succulent, or ornamental plant cell.

12. The host cell of claim 11, wherein said plant cell is a corn, rice, tobacco, alfalfa, soybean, sorghum, potato, tomato, flax, canola, sunflower, cotton, flax, kapok, wheat, oat, barley, or rye cell.

13. The host cell of claim 12, wherein said plant cell is a corn, rice, tobacco, alfalfa, soybean, canola, sunflower, cotton, or wheat cell.

14. The host cell of claim 8, wherein said gene is introduced into said cell by a recombinant vector, virus, cosmid, phagemid, phage, or plasmid.

15. The host cell of claim 8, wherein said gene is introduced into said cell by electroporation, transformation, conjugation, microprojectile bombardment, direct DNA injection, or transfection.

16. The host cell of claim 8, wherein said modified Cry3B* protein comprises the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, or SEQ ID NO:108.]

17. The host cell of claim [16] 8, wherein said modified Cry3B* protein is encoded by the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:99, or SEQ ID NO:107.

18. The host cell of claim 8, wherein said cell is comprised within a transgenic plant.

19. The host cell of claim 18, wherein said cell produces a polypeptide having insecticidal activity against Colorado potato beetle, corn borer or corn rootworm.

20. The host cell of claim 19, wherein said cell produces a polypeptide having insecticidal activity against Leptinotarsa decemlineata, Diabrotica undecimpunctata howardi, Diabrotica virgifera, or Diabrotica virgifera virgifera.

21. A transformed plant cell comprising a modified cry3B* gene that encodes a modified Cry3B* polypeptide having an insecticidal activity which is enhanced against a target insect, compared to a plant cell comprising a native or unmodified cry3B gene, wherein said modified Cry3B* polypeptide comprises the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, or SEQ ID NO:108.

22. The transformed plant cell of claim 21, wherein said modified cry3B* gene produces a modified Cry3B* polypeptide which has enhanced insecticidal activity against Colorado potato beetle, corn borer or corn rootworm.

23. A method of preparing a transgenic plant resistant to infestation by a Coleopteran insect, [comprising] wherein the method comprises transforming said plant with a nucleic acid segment that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, and SEQ ID NO:108.

24. A method of killing a Coleopteran insect, [comprising] wherein the method comprises providing to said insect one or more plant cells transformed with a nucleic acid segment that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, and SEQ ID NO:108, wherein said insect is killed by ingesting one or more of said transformed plant cells.

25. A method of preparing a plant seed resistant to Coleopteran insect attack, said method comprising the steps of:

(a) transforming a plant cell with a nucleic acid segment comprising a modified cry3B* gene that encodes an

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amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:100, and SEQ ID NO:108 to produce a transformed plant cell;

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- (b) producing a transgenic plant from said transformed plant cell; and
- (c) obtaining from said transgenic plant, a seed resistant to attack by said Coleopteran insect.

26. The method of claim **25** wherein step (a) comprises transforming said plant cell by electroporation, protoplast generation, direct transfer of DNA into pollen or an embryo, Agrobacterium-mediated transformation, particle bombardment, or microprojectile bombardment.

27. The method of claim **25**, wherein step (b) comprises generation of plant cells from said transformed plant cell, and regeneration of said transgenic plant from said plant cells.

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